

TECHNIQUES

A simple immunoenzymatic method for measuring IgE in human sera

J. L. GUESDON, R. THIERRY* & S. AVRAMEAS *Unité d'Immunocytochimie, Département de Biologie, Moléculaire, Institut Pasteur Paris, and Laboratoire de Bactériologie de la Faculté de Médecine, Université Louis Pasteur, 67000 Strasbourg, France*

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SUMMARY

A reliable and relatively simple method for the estimation of serum IgE by single radial immunodiffusion is described. The method requires glucose oxidase-labelled antibodies. The method permits the measurement of IgE concentrations ranging from 20 to 700 i.u./ml. The values obtained in unknown samples were in good agreement with those obtained by radioimmunoassay (correlation coefficient $r = 0.9557$).

INTRODUCTION

The technique of simple radial immunodiffusion described by Mancini, Carbonara & Heremans (1965) and by Fahey & McKelvey (1965) is widely used for quantification of a great number of antigens in body fluids but this test is not sufficiently sensitive for measuring IgE concentration in sera or secretions. Rowe (1969), Arbesman *et al.* (1971) and Jalanti & Henney (1972) were able to increase the sensitivity of single radial immunodiffusion by using radiolabelled immunoglobulins. Another test described by Centifanto & Kaufman (1971) uses a fluorescein-labelled immunoglobulins reagent. Although these tests are sensitive and suitable for research studies, they are impractical for routine diagnostic measurements of IgE.

In the present paper we describe the application of an immunoenzymatic technique (Guesdon & Avrameas, 1974), which has been shown to increase twenty-fold the sensitivity of the single radial immunodiffusion technique for the measurement of human IgE. The method was found to allow the measurement of IgE concentrations ranging from 20 to 700 i.u./ml.

MATERIALS AND METHODS

IgE protein. Serum from patient Yu suffering from IgE myelomatosis (Stefani & Mokeeva, 1972) was obtained through the courtesy of Dr Nezlin. Immunoglobulin E was isolated from this serum according to Nezlin *et al.* (1973).

A WHO reference serum, code no. 69/204 containing IgE (10,948 i.u./ml) was supplied by Dr Rowe. Sheep immunoglobulins (gamma-globulins Fraction II) were purchased from Miles Laboratories.

Antisera and antibodies. Antiserum to human IgE was prepared by injecting a sheep with IgE myeloma protein Yu, following an immunization procedure already described. The antiserum was rendered monospecific by absorption on insolubilized normal human serum (Avrameas & Ternynck, 1969) containing low levels of IgE. Specificity of the absorbed antiserum was determined by immunoelectrophoresis, double diffusion and single diffusion. Antiserum to sheep immunoglobulins (Ig) was obtained by injecting rabbits with Ig following an immunization procedure already described (Avrameas, 1969). Antibodies directed against sheep Ig were isolated from this antiserum by using sheep immunoglobulins coupled to polyacrylamide beads (Ternynck & Avrameas, 1972).

Coupling of antibodies with glucose oxidase. The isolated antibodies were conjugated with *Aspergillus niger* glucose oxidase (Boehringer-Mannheim, Germany, grade I, 210 u/mg), using glutaraldehyde as the cross linking agent (Avrameas, 1969).

Correspondence: Dr J. L. Guesdon, Unité d'Immunocytochimie, Département de Biologie Moléculaire, Institut Pasteur, 25 Rue du Dr. Roux, 75015 Paris, France.

After coupling, the preparation was filtered through 0.22 μm sterile Millipore filter and stored at 4°C, or at -20°C in 50% glycerol.

Staining of the slides. Histochemical staining of glucose oxidase on dried slides was carried out for 1 hr at 37°C in the dark using a tetrazolium salt reagent (Avrameas, 1969).

Selection of human sera and determination of the IgE levels by radioimmunoassay. The human sera were chosen randomly among samples sent by the Department of Dermatology and the Department of Allergology of the University Hospital of Strasbourg; determinations were made double blind.

The radioimmunoassay of IgE was made with commercially available Radio-Immuno-Sorbent Test (RIST) from Pharmacia (Uppsala).

Single radial immunodiffusion by immunoenzymatic staining (enzymuno-plates). The whole sheep antiserum to human IgE was diluted in buffered saline solution (BSS) double concentrated (0.30 M NaCl, 0.02 M potassium phosphate buffer, pH 7.4, 1% bovine serum albumin (BSA) and Merthiolate 1/2500 as preservative). An aliquot of this solution was mixed with an equal volume of 2.0% melted agarose (Industrie Biologique Française) in distilled water. The contents of the tubes were mixed by inversion and allowed to stand a few minutes at 48°C in a water bath. The mixture was then poured between two glass plates (8.5 $\times$ 10 cm) separated by 1.5 mm with a frame. Before use one of the plates was coated with 2% agarose and dried at 100°C; the other one was siliconized with Rhodorsil (Rhône-Poulenc). This gel was allowed to solidify and the siliconized plate was gently removed by sliding. Twelve wells 4.5 mm in diameter were cut into the agarose gel using a cork-borer.

To obtain a standard curve, six of these wells were filled with appropriate dilutions of WHO reference serum (20 μl) made in 0.5% BSA. Various sera (20 μl), whose IgE values were to be measured, were placed in the remaining wells. Diffusion was allowed to proceed for 2 days at room temperature in a moist chamber. The slides were washed for 18 hr at 4°C with a large amount of BSS and then incubated face down in glucose oxidase-labelled antibody preparation (5–10 μg of antibody per ml of BSS containing 0.5% BSA). One hour later the incubation medium was discarded and the slides were post-incubated overnight at room temperature in a moist chamber. The slides were then washed for 24 hr at 4°C with several changes of BSS, dried under filter paper and stained for enzymatic activity.

RESULTS AND DISCUSSION

To determine the optimal conditions for measuring IgE, agarose plates containing various dilutions of sheep anti-human IgE antiserum were prepared and dilutions of reference serum were placed in the wells. The clearest diffusion rings were obtained with a 1:1500 dilution of this antiserum. This dilution was chosen for the preparation of the plates used in the determination of IgE present in the various sera. The smallest IgE concentration which could be easily determined was 40 i.u./ml. In the conditions used (antiserum dilution 1:1500, diffusion time 48 hr and wells of 4.5 mm), when the precipitin ring diameter squared was plotted against IgE concentration, the relationship obtained was linear between 40 i.u./ml and 700 i.u./ml. The experimental points fell on the theoretical regression line with an error value lower than 4%. Repeated estimations of the IgE concentration on different days with different plates showed variations of less than 10%. By using higher dilutions of antiserum incorporated in the agarose gel 20 i.u./ml of IgE could be determined.

The IgE levels of 100 samples of human sera were determined by radioimmunoassay and by the present immunoenzymatic method. The two populations of values obtained are reported in Table 1 and compared. Because the distribution of IgE levels was logarithmico-normal (Thierry *et al.*, 1975) the values have been replaced by their logarithms to allow the utilization of the classical statistical methods. The *F* test (*F* = 1.059) shows that the two populations are statistically identical. (The probability that the observed value of *F* is greater than 3.89 is exactly 0.05.) Furthermore the correlation is strongly demonstrated by the correlation coefficient (*r* = 0.9557) and is illustrated by the tolerance ellipse (*P* = 0.01) shown in Fig. 1, where the logarithms of IgE levels are used. The greater divergence occurs with the higher levels; when the IgE concentration exceeds 2000 i.u./ml the radioimmunoassay gives an over-estimation probably due to the extrapolation shown in the sigmoidal part of the standard curve.

In conclusion it appears that both radio-immunoassay and Enzymmuno-plates give comparable results. The immunoenzymatic method is less sensitive than radio-immunoassay but the sera containing low IgE levels (< 40 i.u./ml) are not numerous and their clinical interest is limited. Moreover the Enzymmuno-plates allow the titration of IgE in short series of sera and they do not require elaborate and expensive materials.

TABLE 1. Determination of serum IgE concentration (IU/ml) by radioimmuncassay and by immuncenzymatic method

Sample no.	Method		Sample no.	Method	
	Immunoenzymatic method	Radioimmunoassay		Immunoenzymatic method	Radioimmunoassay
1	165	209	51	176	202
2	286	440	52	1320	1507
3	153	256	53	1760	1859
4	880	1805	54	114	162
5	220	249	55	242	375
6	660	748	56	451	455
7	1452	1406	57	62	50
8	72	77	58	22	43
9	572	935	59	149	139
10	3080	5250	60	2569	3906
11	40	36	61	523	471
12	898	2218	62	149	134
13	295	284	63	431	394
14	40	47	64	123	135
15	124	180	65	182	220
16	220	335	66	480	359
17	236	184	67	45	106
18	1724	3125	68	220	215
19	181	287	69	343	424
20	492	459	70	376	336
21	1320	1265	71	334	333
22	506	671	72	209	206
23	70	60	73	1760	8000
24	642	636	74	184	158
25	880	2156	75	143	265
26	216	249	76	369	314
27	528	558	77	1953	4750
28	444	383	78	81	99
29	84	119	79	253	310
30	88	120	80	91	83
31	72	73	81	2956	4000
32	154	208	82	158	157
33	290	278	83	338	672
34	66	130	84	678	2515
35	182	295	85	426	371
36	72	107	86	836	713
37	97	138	87	1548	2562
38	66	124	88	299	279
39	713	1103	89	84	96.5
40	224	270	90	361	318
41	176	264	91	356	278
42	72	134	92	827	1703
43	71	88	93	132	171
44	301	268	94	915	1812
45	182	205	95	418	349
46	275	287	96	110	106
47	448	467	97	1350	1687
48	440	393	98	615	613
49	504	486	99	110	125
50	198	200	100	720	660

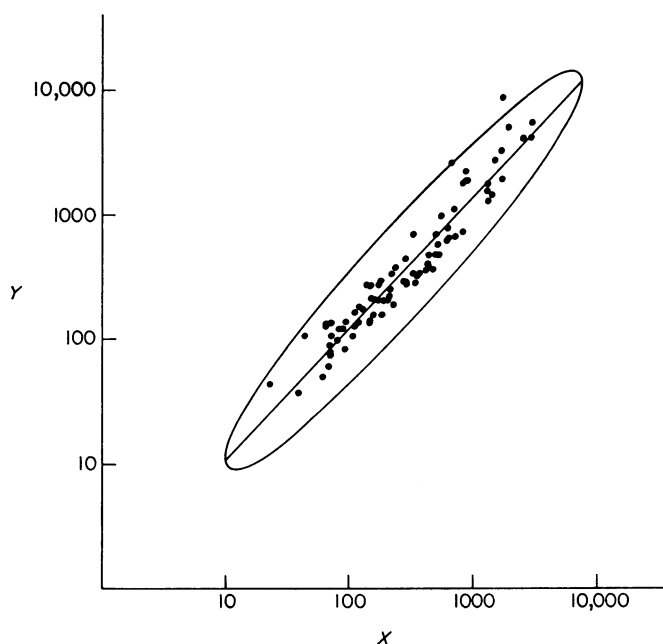


FIG. 1. Comparison between the IgE concentrations (i.u./ml) obtained by Enzymimmuno-plates (X) and by radio-immunoassay (Y). Tolerance ellipse: $P = 0.01$; $F = 4.80$.

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