

A microplate enzyme-immunoassay for toxoplasma antibody

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SYNOPSIS A new test for the detection and measurement of toxoplasma antibody is described. Test sera are reacted with antigen-sensitized wells in micro-haemagglutination plates. Any attached antibody is shown by the addition of an enzyme-labelled antiglobulin followed by assay of the enzyme reaction with its substrate. The test is easy to carry out on a large scale, and there is a positive correlation between the results and dye test and haemagglutination test titres.

The diagnosis of toxoplasmosis is not easy and is usually based on the critical assessment of one or more serological tests. The dye test (Sabin and Feldman, 1948), indirect immunofluorescence (Kelen *et al*, 1962), and passive haemagglutination (Jacobs and Lunde, 1975) are the tests most frequently employed. Karim and Ludlam (1975) have recently devised a system of integrating the results of several tests to give more precise information on the stage of the disease. There is clearly scope for other methods and, since enzyme immunoassays have given promising results in two other protozoal parasitic infections, Chagas disease (Voller *et al*, 1975a) and malaria (Voller *et al*, 1975b), we thought it worthwhile to investigate their potential in toxoplasmosis.

Material and Methods

ANTIGEN

Toxoplasma gondii organisms obtained from peritoneal exudates of infected mice were ruptured by freeze-thawing once followed by sonication for 20 seconds at 4°C. The cell debris was removed by centrifugation at 10 000 g for 30 minutes, and the supernatant fluid (the stock antigen) was stored in aliquots at -70°C until used.

SERA

One hundred and ninety-eight sera submitted to the Public Health Laboratory, St. George's Hospital,

London were tested by the dye test (Sabin and Feldman, 1948), by passive haemagglutination (Jacobs and Lunde, 1975), and by the microenzyme-linked immunosorbent assay (MICRO-ELISA) described below. These sera came from clinically suspected cases of toxoplasmosis and 16 of them with dye test titres over > 1/1024, were from typical lymphadenopathy cases.

MICRO-ENZYME-LINKED IMMUNOSORBENT ASSAY

The enzyme-linked immunosorbent assay (ELISA) of Engvall and Perlmann (1972) with the modification (MICRO-ELISA) of Voller *et al* (1975a) was used. The principle of the assay is described in figure 1. For toxoplasmosis the test was carried out as follows:

- 1 The wells in microhaemagglutination plate (COOKE MICROTITER M29 AR¹) were sensitized with the toxoplasma antigen, diluted in 0.05M carbonate buffer, pH 9.6, overnight at +4°C. The plates were then given three washes each of 3 minutes in phosphate buffered saline (PBS) containing 0.05% Tween 20 and were shaken dry.
- 2 0.3 ml of the diluted serum was added to each well and incubated for 2 hours at room temperature. The plates were washed as before in PBS/Tween and were shaken dry.
- 3 0.3 ml of the diluted antiglobulin conjugate was added to each well and incubated for 3 hours

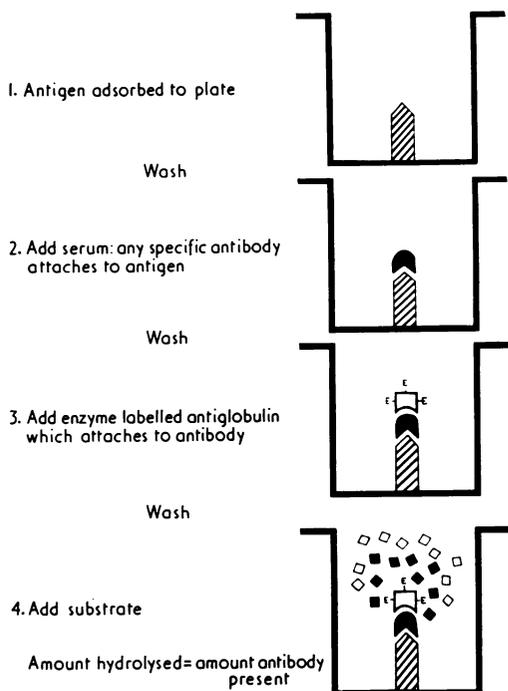


Fig 1 Principle of the MICRO-ELISA technique.

at room temperature after which the plates were again washed and shaken dry. (This conjugate consisted of rabbit antihuman globulin labelled with alkaline phosphatase (Engvall and Perlmann, 1972).)

4 0.3 ml of the enzyme substrate was added (substrate = 1 mg p-nitrophenylphosphate per ml diethanolamine buffer, pH 9.8, containing 0.5 mmol MgCl₂). The enzymic hydrolysis of substrate was stopped after 30 minutes by the addition of 0.05 ml 2M NaOH.

The results are expressed as the absorbance at 400 nm which was determined in a spectrophotometer with a 1 cm light path.

PRELIMINARY TITRATIONS

The antigen preparation was titrated in a checker-board fashion against a positive reference serum sample and against a negative reference sample. An antigen dilution of 1:200 gave the biggest difference between the positive and negative sera, and this was used in all subsequent tests. The positive and negative reference antisera were similarly titrated, and the biggest difference between them was at a serum dilution of 1:200 so this was used in the tests.

Results

Tests on a small batch of sera indicated that an absorbance value of 0.3 or less usually corresponded with negative dye test and IHA results. Accordingly, in the test reported here all absorbance values (\equiv MICRO-ELISA value) over 0.3 have been considered positive.

In table I the relationship of positive and negative MICRO-ELISA values to dye test titres is shown. Table II shows the relationship of positive and negative MICRO-ELISA values to IHA titres.

It can be seen that virtually all sera with dye test titres of 1/64 or above, or IHA titres of 1/40 or above, have positive MICRO-ELISA values. Between 10% and 20% of the sera with negative dye test or IHA had positive MICRO-ELISA values. Comparison of positive and negative results obtained by IHA or dye test showed a similar discrepancy of 10-20% between the tests.

The mean MICRO-ELISA values for the different dye test titre groups and IHA titre groups are given in figure 2. The positive correlation between dye-test, IHA, and mean MICRO-ELISA values is clear. The distribution of MICRO-ELISA values for each dye test titre group and each IHA titre group are shown in figs 3 and 4 respectively.

Dye Test Titre Group	No. of Sera in Group	MICRO-ELISA Results		
		Negative	Positive	% Positive
< 16	91	78	13	14
16/32	17	13	4	23
64/128	29	5	24	83
256/512	34	2	32	94
1024 and above	27	1	26	96

Table I Relationship of toxoplasma dye test results to MICRO-ELISA results (the latter are grouped as positive (> 0.3) or negative (\leq 0.3))

IHA Titre Group	No. of Sera in Group	MICRO-ELISA Results		
		Negative	Positive	% Positive
< 40	106	87	19	18
40/80	39	9	30	77
160/320	31	2	29	94
640/1280	16	1	15	94
Above 1280	6	0	6	100

Table II Relationship of toxoplasma passive haemagglutination results to MICRO-ELISA results (the latter are grouped as positive (> 0.3) or negative (\leq 0.3))

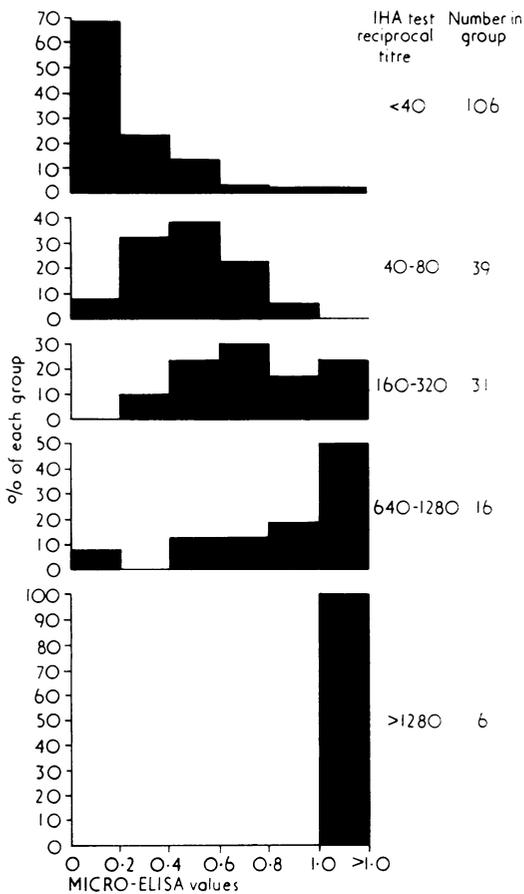


Fig 2

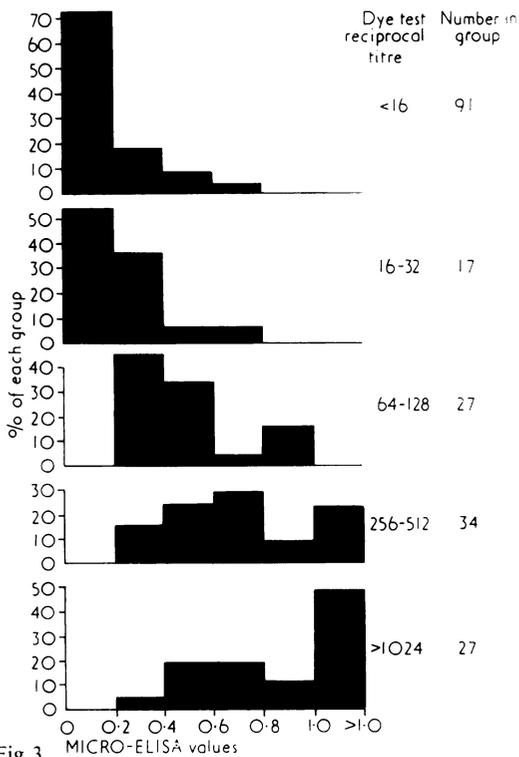


Fig 3

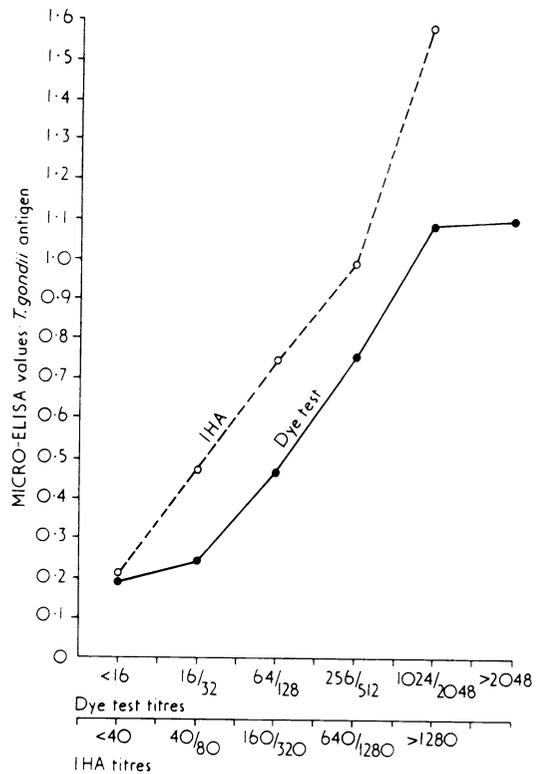


Fig 4

Fig 2 Mean MICRO-ELISA values for the sera grouped according to IHA titres (dotted line) or dye test titres (solid line).

Fig 3 Distribution of MICRO-ELISA values of sera grouped according to dye test titres.

Fig 4 Distribution of MICRO-ELISA values of sera grouped according to IHA titres.

Discussion

Enzyme immunoassays are inherently attractive as they are sensitive, give only objective results, and are suitable for large-scale automation. The reagents used are stable and, although enzymes are expensive, only minute quantities are used so that the cost per assay is low. There are, however, difficulties in standardizing the test, and small changes in procedure may lead to large differences in end results. The present methods for preparing the conjugates are considered inadequate as only a small proportion of the globulin is labelled, and unless the preparation is carefully fractionated unlabelled globulin and free enzyme are present.

However, in spite of these difficulties the preliminary results on toxoplasma suggest that enzyme immunoassays may take their place alongside other established methods. The results show that, overall, there is a positive correlation between dye-test and MICRO-ELISA values and IHA and MICRO-ELISA values, although there are a few notable exceptions where high titred sera in one test gave low values in the other. The correlation between IHA and MICRO-ELISA values was better than that between dye-test and MICRO-ELISA.

Perhaps a more serious disadvantage which emerges is that the ELISA, carried out as described, does not adequately discriminate between sera with medium dye-test titres (eg, 256-512) and those with very high titres (eg, over 1024). This distinction is clinically important. Possibly the use of purified antigens and better conjugates will enable this differentiation to be made. In the present study only an anti-immunoglobulin conjugate has been employed, but there is no reason why class specific im-

munoglobulin conjugates should not be used, in which case it would be possible to detect specific IgM toxoplasma antibodies.

These preliminary studies indicate the potential of the enzyme immunoassay method for diagnosis of toxoplasma infection, and we feel that they justify further investigation.

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