Enzyme-linked immunosorbent assay for the serodiagnosis of toxoplasmosis

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SUMMARY The enzyme-linked immunosorbent assay (ELISA) for toxoplasmosis proved as sensitive as the dye test and indirect haemagglutination (IHA). The reproducibility of end-point titres was better than that of the extinction values obtained from a single serum dilution. In a comparison of 152 sera, ELISA was found to correlate better with IHA than with the dye test. The use of ELISA for routine serology and population screening is discussed.

Since its introduction by Engvall and Perlmann,¹ the enzyme-linked immunosorbent assay (ELISA) has been used to detect antibodies in a variety of infectious diseases. Several investigators have evaluated ELISA for the serodiagnosis of toxoplasmosis.²⁻⁷ Different authors have found the correlation with the indirect immunofluorescence (IIF) and dye test, and with indirect haemagglutination (IHA), to vary, so that the value of ELISA in toxoplasmosis serology has been difficult to assess. Our particular interest was whether ELISA might replace one of the established methods, and whether it could be used as a screening test.

Material and methods

ANTIGEN

Toxoplasma gondii cells (RH strain) were harvested from the peritoneal exudate of infected mice and filtered (Schleicher u. Schüll, Dassel, No. 1450). The cells were collected by centrifugation at 3000 *g* and washed three times in phosphate buffered saline (PBS). The sediment was resuspended in 4 ml PBS and sonicated (300 S Ultrasonic, Braun Melsungen) at 4°C for 7 minutes. After centrifugation at 10 000 *g* for 30 minutes, the supernatant (soluble antigen) was stored in aliquots in liquid nitrogen.

SERA

Sera submitted for toxoplasmosis serology are examined routinely by the dye test, complement fixation test (CFT), and indirect haemagglutination (IHA); 152 serum samples showing different serological patterns were examined by the micro-

ELISA technique. These sera were divided into three groups on the basis of the dye test and CFT titres, as follows:

- Group 1 dye test < 1/16 (negative)
- Group 2 dye test titre between 1/16 and 1/1000, CFT titre ≤ 1/10 (intermediate titres)
- Group 3 dye test titre ≥ 1/1000, CFT titre > 1/10 (high titres)

CONJUGATE

Commercially available horseradish peroxidase conjugated sheep antihuman globulin (Institut Pasteur) was used for the ELISA.

SUBSTRATE

This was prepared as follows: 80 mg of 5-amino-salicylic acid were dissolved in 100 ml of distilled water (80°C). Immediately before use, the pH was brought to 6.0 with 1 *N* NaOH. Nine parts of the above solution were mixed with one part of 0.05% H₂O₂.

MICRO-ELISA

The test was carried out essentially as described by Voller *et al.*⁶ Flat-bottomed polystyrene microtitre plates (Cooke M 29 A, Dynatech Laboratories) were coated with 0.15 ml toxoplasma antigen, diluted to optimal concentration in 0.05 *M* carbonate buffer (pH 6.0), and left overnight at + 4°C. Before use the plates were washed again, and 0.15 ml of the conjugate, diluted in PBS/Tween, was added to the wells. The plates were incubated for 1 hour at 37°C and then washed as before. Finally, the wells were filled with 0.15 ml of the substrate, and the plates were left at room temperature for 30 minutes. The enzyme reaction was stopped by adding 0.05 ml 1 *N*

NaOH to each well, which was then read with a spectrophotometer (Titertek Multiskan, Flow Laboratories) at 450 nm. The last serum dilution yielding a higher extinction value than the 1 in 16 dilution of the negative control serum was regarded as the end-point. Ninety additional sera were also tested at a 1 in 100 dilution; the results are expressed as extinction values (E_{450}).

PRELIMINARY TEST

The optimal antigen and conjugate concentration was established by chess-board titration against a known positive and negative reference serum. The dilution showing the greatest difference between extinction values of the positive and negative sera was used for the assay.

Results

RELATIONSHIP OF ELISA TITRES TO E_{450} VALUES OF A SINGLE SERUM DILUTION

In a preliminary study the ELISA end-point titres of 67 sera were compared with the E_{450} values of a 1 in 100 dilution of the same sera. The results are plotted in Figure 1. The correlation coefficient of 0.92 ($P < 0.001$) indicated that the antibody titre of a serum could be determined from the E_{450} value of the 1 in 100 dilution.

Fig. 1 Relationship of the ELISA end point titres to the E_{450} values at a 1 in 100 dilution in 67 serum samples.

REPRODUCIBILITY

The reproducibility of the ELISA was tested by performing 10 assays on different days with the positive reference serum. The titre end-points and E_{450} values of the 1 in 100 serum dilution were determined.

From the E_{450} values the titres were also calculated using the regression line in Figure 1. All 10 tests gave end-points of 1/2000. The E_{450} values of the 1 in 100 serum dilution ranged from 1.143 to 1.59. The calculated titres differed only by ± 1 twofold dilution.

The positive reference serum was also tested in a 1 in 100 dilution in each well of two microtitre plates on the same day. The arithmetic mean of the E_{450} values of each plate was taken and $\pm 10\%$ was allowed for experimental error. Only two readings exceeded the $\pm 10\%$ limit on plate I, whereas 19 readings did so on plate II.

COMPARISON OF ELISA, DYE TEST, AND IHA TITRES

End-point titres of 152 serum samples obtained by the ELISA technique were compared with the dye test and IHA titres (Figs 2 and 3). With a range of ± 1 dilution step there was an agreement of 78% between ELISA and the dye test and of 89% between ELISA and IHA.

Fig. 2 Comparison of ELISA and dye test titres in 152 sera.

Four sera (2.6%) which were negative in the dye test ($< 1/16$) gave positive results with ELISA, yielding titres of 1/256, 1/64, and 1/16 twice. The same percentage of sera gave false negative reactions in ELISA.

In group 1 sera, which were nonreactive in the dye test and CFT, ELISA correlated well with the dye test (94%) and with IHA (88%). Group 2 sera, giving intermediate titres, correlated well with ELISA and the dye test in 90% and with ELISA and IHA in 87% of cases. In the high-titre group 3 sera,

Fig. 3 Comparison of ELISA and IHA titres in 152 sera.

however, ELISA titres were much lower than dye test titres. The dye test agreed with ELISA in only 53% of cases, in contrast to a good correlation (89%) between ELISA and IHA (Table).

Discussion

The reproducibility of the micro-ELISA for toxoplasmosis was found to be satisfactory. Determination of the end-point was more accurate than measurement of E_{450} values of a single 1 in 100 serum

Percentage of agreement within ± 1 dilution step between ELISA and the dye test and between ELISA and IHA for sera grouped according to their dye test and CFT titres

Group*	Percentage of agreement between:		
	ELISA/dye test	ELISA/IHA	n
All sera	77	89	152
1	94	88	47
2	90	87	62
3	53	89	33

*1 = negative, 2 = intermediate titres, 3 = high titres.

dilution. As there are considerable variations in the quality of microtitre plates, this source of error would play a more important role in determining the E_{450} value than the end-point titre.

The present investigation has shown that the antibody titre obtained by ELISA correlates better with IHA than with the dye test titres, as also described by Voller *et al.*⁶ This seems to be based on the fact that ELISA and IHA utilise soluble antigen, whereas the dye test and IIF are performed with whole intact parasites. In the early stages of the infection, antibodies to the cell wall are the first to be formed. The discrepancy between ELISA and the dye test is, therefore, particularly marked in sera presenting higher dye test titres. Similar results have been reported by Voller *et al.*⁶ and also by Ruitenber and van Knapen,⁵ who compared ELISA and IIF. Other authors,^{3,7} however, claim a close correlation between ELISA and IIF. These differing results may be due to a lack of standardisation of the ELISA

Fig. 4 Relationship of the ELISA E_{450} values at a 1 in 100 serum dilution to the corresponding dye test titres of 90 sera. The significance limits of extinction values to be used in a screening test for toxoplasmosis might be set at 0.25 (dye test titre $> 1/16$) or at 0.5 (dye test titre $> 1/64$).

method, in particular in the preparation and purification of the antigen.

The question arises whether ELISA could replace one of the established tests in toxoplasmosis serology. The sensitivity of ELISA is comparable to that of the dye test and IHA but is not higher. Since ELISA as used here does not discriminate between sera with intermediate and high dye test titres it cannot be used as an alternative to the dye test or IIF.

ELISA and IHA seem to measure the same type of antibodies. Each method has its advantages. IHA can be performed easily and quickly, and no additional equipment is required. For testing large numbers of sera, ELISA has the advantage of possible mechanisation.

ELISA may be useful in seroepidemiology. In Fig. 4, the ELISA E_{450} values of 90 sera are plotted against the corresponding dye test titres. On the basis of the interpretation of the significance limits of dye test titres ($>1/16$ or $>1/64$), a certain E_{450} value must be set for the detection level. The examples in Fig. 4 show that a small percentage of false negative (2-3%) and false positive (4-6%) results is unavoidable.

We consider that ELISA brings no clear advantage in the routine serological diagnosis of toxoplasmosis but would be useful in population screening if the method were standardised.

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