One Point Dilution Enzyme-Linked Immunosorbent Assay (ELISA) for Toxoplasma gondii Seroepidemiological Surveys

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A one point dilution enzyme-linked immunosorbent assay (ELISA) procedure suitable for determining immunoglobulin G (IgG) antibody levels to Toxoplasma gondii (T. gondii) in community seroepidemiological surveys is described. A two-fold serial dilution ELISA procedure was first used to determine the IgG titers in 56 and 83 sera earlier screened by the Sabin-Feldman dye test (DT) and the indirect hemagglutination test (IHA), respectively. The regression rate of the results by the DT and ELISA was 0.92. Comparison of the results by the IHA and the two-fold serial dilution ELISA gave regression coefficient of 0.92. Using the absorbance values for the test sera at dilutions of 1:20, standard curves made by plotting the optical density versus the corresponding dilution factor of a control sera were used to estimate the antibody levels. The regression coefficient of the results by the two-fold serial dilution method and those by the curves for sera with titers of up to 1:320 was 0.97. The curves could not, however, estimate accurately the antibody level in sera with titers above 1:320. The one point dilution ELISA described is a useful epidemiological tool for the screening of IgG antibody to Toxoplasma gondii in the community. However, larger series are required to confirm our observations. ——— Toxoplasma gondii; ELISA; seroepidemiological survey

A number of serological tests have been described for the detection of antibody to Toxoplasma gondii. In choosing a diagnostic test, however, the specificity, sensitivity, safety, cost and time required to get the results are some of the major determining factors. In this regard, the enzyme linked immunosorbent assay (ELISA), has been noted to offer a combination of the best qualities (Wisdom 1976). Although originally described as a single tube, photometrically
quantitated procedure (Clamp et al. 1971), ELISA was rapidly adapted to microtitration and automation (Ruiternberg et al. 1977). Therefore ELISA provides quantitative results and eliminates the subjectivity associated with other tests presently in use. However, the failure to use one point dilution has been a major obstacle in using the procedure for screening a large number of samples within a short time. van Loon and van der Veen (1980) reported a single dilution ELISA in which they used an initial serum dilution of 1: 800. These workers were able to determine the IgG antibody level in sera from patients with features suggestive of toxoplasmosis.

In epidemiological surveys, however, the single dilution ELISA as described by these workers cannot be useful since it is unable to detect titers less than 1: 800. Furthermore, their method requires mathematical calculations. We describe a one point dilution ELISA procedure which permits a precise and reproducible quantization of IgG antibodies to T. gondii. The sensitivity of the test was compared with that of DT and the IHA. Results by the one point dilution are also compared with those by the serial two fold dilution ELISA.

**MATERIALS AND METHODS**

Antigen. The antigen was prepared from the Rh strain of T. gondii using the method described by Bodner et al. (1972).

ELISA procedure. The micro-ELISA technique by Voller et al. (1976) with some modification was used. The test was performed as described below: (a) The microplates were coated with Toxoplasma antigen diluted in 0.05 M carbonate buffer pH 9.6 overnight at 5°C (The antigen protein concentration was 40 μg/ml as determined by the method of Lowry and coworkers). The plates were then washed with phosphate buffer solution (PBS) containing 0.05% Tween 20 on a magnetic stirrer. The exercise was repeated three times each wash lasting for 3 min. The plates were then shaken dry and used or stored at −20°C until use. (b) Diluted serum, 100 μl, was then added to each well and incubated in a humidified chamber at 37°C for 60 min. Subsequently the plates were washed with PBS Tween as before and shaken dry. (c) Diluted alkaline phosphatase, 100 μl, labeled goat IgG fraction against human immunoglobulins were next poured into each well followed by incubation for 1 hr at 37°C in a humidified chamber. Thereafter the plates were washed and shaken dry as described before. (d) The substrate, 100 μl, prepared by adding 1 mg of p-nitrophenylphosphate to 1 ml of diethanolamine buffer, pH 9.8 were poured into each well and incubated at room temperature for 30 min at the end of which the optical density of each well was determined by the MR 580 micro-ELISA automatic reader, (Dynatech Instruments Inc. Santa Monica, Ca., USA). The optical densities were determined using 405 nm and 490 nm as the major and minor filters respectively. Only optical densities greater than three times that of negative control for the corresponding dilution were considered significant.

Determination of optimum times of incubation and antigen concentration. In preliminary titrations, various antigen concentrations had been titrated against a positive and a negative serum samples in a checkerboard fashion. The two reference sera had titers of 1: 256 and 1: 4 as determined by the dye test method. Antigen dilution of 1: 100 (0.4 μg/100 μl) and incubation time of 1 hr were found to be optimum and were thus used in all the subsequent experiments.

Sera. The IHA or the DT are routinely used for the screening of sera for toxoplasma antibody at the Institute for Tropical Medicine, University of Nagasaki. Fifty six sera
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previously screened by the DT and 83 sera screened by IHA method were examined using the ELISA procedure described above. The control sera were in all occasions run together with the test sera. Standard curves were made by plotting optical density values against the corresponding serial dilution of the control sera.

Regression coefficient. For the calculations of the regression coefficient values in this study, antibody titers of less than 1: 4 for the DT, less than 1: 80 for the IHA and less than 1: 20 for the ELISA were assumed to have a value of 1.

RESULTS

Reproducibility of ELISA

To test the reproducibility of the serial two-fold dilution ELISA each of the seven serum samples with different antibody concentrations was run 14 times within the same test. The standard deviation of the mean optical density values for each serum dilution were not more than 0.1 except at a dilution of 1: 40 for one sample where the mean standard deviation was 0.468. The results indicate a high degree of reproducibility.

Correlation between DT and ELISA

Comparative data in Fig. 1 shows that there was agreement by the two tests in 46 (82%) samples. In the remaining 10 samples ELISA test was positive but DT was negative in all of them. The regression rate of the results by the two tests was 0.92.

Correlation between ELISA and IHA

Of the 83 sera analyzed by IHA 47 were positive for antibody to T. gondii. When the 83 samples were screened by the two fold dilution ELISA procedure 25 were negative and 57 were positive. In one IHA negative sample the ELISA test

![Fig. 1. Correlation between titers obtained by DT and ELISA technique for 56 sera.](image-url)
was negative in all dilutions except 1:80 and 1:160. This sample was excluded in the subsequent analysis of the data. ELISA test results for the remaining 82 sera were then compared with those by the IHA method, as shown in Fig. 2. Of the 36 sera negative by IHA, 25 (69%) were also negative by ELISA and in 10 samples the test was positive at low dilutions (less than 1:160). The regression coefficient between the IHA and ELISA test readings was 0.92. Comparative data for 47 IHA positive sera were as shown in Fig. 3. In 13 samples (28%) there was complete agreement between the two tests. In 37 sera (79%) the agreement was within ±1 two point dilution. Agreement within ±2 two point dilution was 96%. In 2 sera (4%) results by the two tests differed by ±3 two point dilution.

**One point dilution ELISA**

The regression coefficient of the results by one point dilution and those by the two fold serial dilution ELISA was over 0.97 for the sera with titers up to 1:320. These results are shown in Fig. 4.

**DISCUSSION**

The ELISA described in the present study was found to be highly reproducible. Similar observations on the reproducibility of ELISAs have been reported by other workers (Walls et al. 1977; Lin et al. 1980; Woodward 1982). The agreement between results by one point dilution and those by the two fold serial dilution ELISA in sera with titers of up to 1:320 was good as evidenced by a...
regression coefficient of 0.97. Determination of final titers by one point dilution was unreliable for sera with titers higher than 1:320. Reports on the use of standard curve obtained from serial dilutions of control sera for assay of antibodies to viral diseases has been published (Abu Elzein and Crowther 1978). That there was agreement between DT and ELISA in 82% of the samples tested.
is significant. The discrepancy which occurred in ten samples can not completely be accounted for. Noteworthy however, is the fact that whereas the DT detects antibodies directed to the toxoplasma cell wall only; ELISA probably detects antibodies directed to the cell wall as well as antibodies directed to the soluble antigens of the parasite (Carlier et al. 1980). This may at least in part account for the higher positivity by ELISA compared to DT. Similar discrepancies have been reported between DT and IHA (Voller et al. 1976). Positive correlation between DT and ELISA has also been reported by earlier workers (Voller et al. 1976; Lin et al. 1980; Woodward 1982).

On the correlation between results by ELISA and IHA by and large our results agree with those published by other workers (Voller et al. 1976; Lin et al. 1980; Woodward 1982). In a few serum samples IHA gave higher titers than ELISA. More frequently however, the antibody titer tended to be higher with ELISA than IHA. In addition 10 IHA negative samples were positive by ELISA at low dilutions. ELISA test is reported to give false positive results in sera with antinuclear antibodies (ANA) or rheumatoid factor (RF) (Walls et al. 1977). However, in spite of lack of accurate data on the incidence of RF and ANA in Japan, antinuclear antibodies in the population are very rare (Takehara et al. 1985). Moreover, it has been reported that although the RF may affect the degree of positivity detected for a given sample it does not affect positivity itself (Woodward 1982). Therefore these results are unlikely to have been affected significantly by presence of ANA and/or RF. Since the IHA and ELISA detects antibodies directed to the toxoplasma cell wall as well as those directed to the soluble antigens of the parasite the discrepancy between the results by the two tests need to be explained. That ELISA gave higher positive titers than IHA in 24 out of 47 sera (Fig. 3), is probably a reflection of several factors. On the first place the discrepancy could be attributed to differences in the antigenic determinant groups being detected. Secondly, in recent infections ELISA gives higher IgG titers than IHA (Camargo et al. 1978). It is also tenable that a larger number of antigenic components are adsorbed to plastic surfaces than to the acid treated red blood cells used in IHA. The determination of the end point by special equipment in ELISA is certainly more sensitive and objective than the macroscopic determination of the end point in IHA. Other factors unknown to us could in part account for the discrepancy.

Many reports that have been published on the role of ELISA in the serodiagnosis of toxoplasmosis indicate that the test could soon acquire a definite place in the serodiagnosis of toxoplasmosis. Furthermore, the mechanization of ELISA as described by Ruiternberg and co-workers (1977) make the test suitable for screening large number of sera per day. However only a few specialized laboratories can make optimum use of such equipment. The one point dilution ELISA procedure described by van Loon and van der Veen (1980) could be useful in determining the antibody levels in sera with titers of at least 1:800. To be
noted, however, is the fact that although toxoplasmosis is very common, occurring in about 50% of the adult population in some communities (Krick and Remington 1978; van der Veen and Polack 1980), the affected individuals in almost all cases are asymptomatic. Furthermore, the antibody levels in these individuals are usually low. The one point dilution ELISA procedure described herein is not only suitable for large scale population screening but could also be used in screening individuals on immunosuppressive drugs or with immunodeficiency syndromes in whom previously acquired toxoplasmosis may get reactivated (Ruskin and Remington 1976; Krick and Remington 1978). Also new infections in these individuals often lead to disseminated disease (Carey et al. 1973; Krick and Remington 1978). In most of these patients however, the IgG antibody levels will rise in the event of reactivation. In new infections IgM and then IgG antibodies to *T. gondii* would be detected since the humoral immunity in such patients is usually not affected.

References


