COMBINATIONS OF THREE IMMUNOLOGICAL ASSAYS FOR DETECTING ANTI-*TOXOPLASMA* IgG IN THE SERA OF PATIENTS INFECTED WITH *TOXOPLASMA GONDII*

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Abstract. Enzyme-linked immunosorbent assay (ELISA), Dot-ELISA and Dot-immunogold silver staining (Dot-IGSS) were simultaneously used to detect the specific IgG against *Toxoplasma gondii* in 65 patients infected with the protozoa. The positive rates were 86.51%, 92.51% and 98.64%, respectively. When ELISA and Dot-ELISA results were put together, the positive rate increased to 95.38%. When Dot-IGSS results were combined with those of ELISA or Dot-ELISA, the positive rate was raised to 100%. The difference in positive rate between ELISA and Dot-IGSS was significant ($x^2 = 6.93$, $p < 0.01$), but no statistically significant differences were found between ELISA and Dot-ELISA or between Dot-ELISA and Dot-IGSS. Paired comparison of the reacting intensities of the sera in the 3 assays showed the correlations were highly significant ($p < 0.001$), with $r = 0.608$ between Dot-IGSS and Dot-ELISA, $r = 0.8194$ between Dot-IGSS and ELISA and $r = 0.517$ between Dot-ELISA and ELISA. Hence combination of different serological assays may increase their sensitivity and specificity for detecting the anti-*Toxoplasma* antibodies.

INTRODUCTION

Toxoplasma gondii infection is common in China and the infective rate was reported to be 5.17% (0.33-11.97%) (Cui, 1991). Toxoplasmosis is a parasitic zoonosis and may transmitted in many modes. The infection is usually latent and when the host's immune function becomes reduced, serious clinical symptoms appear. Moreover it is important to notice that *Toxoplasma gondii* can infect newborns through placental transmission. We know that parasitological diagnosis is important, but it is very difficult to make and takes time. Although detection of circulating antigen of the protozoa or anti-*Toxoplasma* IgM in the patient's serum is rational, the test can only be made in a few laboratories, where sophisticated facilities are available. Hence detection of anti-*Toxoplasma* IgG remains as the common means for diagnosing toxoplasmosis. The methods frequently used include indirect hemagglutination test (IHA), indirect fluoresecent antibody test (IFAT) and enzyme linked-immunosorbent assay (ELISA). Among the 3 methods, ELISA had been recommended as the best one. It was also advocated that in order to increase the accuracy and reliability of diagnosis, 2 or more serological tests be carried out simultaneously, laboratory conditions permitting (Editorial Committee, 1991). In this present experiment, we tried to complement ELISA by making Dot-ELISA and Dot-immunogold silver staining (Dot-IGSS), so as to develop a more suitable for accurate diagnosis of toxoplasmosis.

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MATERIALS AND METHODS

Sera

Sixty-five sera of pregnant women or lying-in women, who were considered to have been infected with *Toxoplasma gondii* by preliminary immunological assay, and 76 control sera of normal pregnant women were collected from Xuzhou Maternity Hospital and the Affiliated Hospital of Xuzhou Medical College.

Antigen of *Toxoplasma gondii*

The soluble antigen of *Toxoplasma gondii* tachyzoite was provided by the Institute of Animal

Husbandary and Veterinary of Jiangsu Academy of Agro-Sciences, with nitrogen concentration being 3.7 mg/ml.

Methods of detection

ELISA: The assay was carried out according to the classical method (Deelder *et al*, 1977). The antigen was diluted in 1:400 with 0.05 mol/l pH 7.4 phosphate buffered saline (PBS) and added into the wells of 40-well flat-bottom polystyrene microreaction plates, with 50 µl per well. The plate was left overnight at 4°C for coating. All sera were dilluted to 1:50 before test. The horseradish peroxidase labeled sheep anti-human IgG (Beijing Institute of Biologicals) was 1:500 dilution. The substrate was phenylenediamine. After the reaction was stopped by adding 2M H_2SO_4, the optical density (OD 490) was read. More than 2 times the control OD was taken to indicate positive reactions.

Dot-ELISA: The technic was developed by modifying Hawkes' method (Hawkes *et al*, 1982). Mixed cellulose ester micropore filter membrane (MCE pore size 0.22 µm) was used to replace nitrocellulose filter paper. A drop of 1:20 antigen (about 1 µl) was added with a pin on to the center of each MCE square. TBS (pH 7.4) containing 10% calf serum was used as the diluting and blocking solution. The positive sera were diluted sequentially from 1:20 to 1:2,560 for the test, while the negative controls were tested at 1:20 dilution. Horseradish peroxidase-labeled sheep anti-human IgG (Beijing Institute of Biologicals) was diluted 1:40 before use. The substrate was 4-chloro-1-naphthol. The positive reaction was decided by the appearance of blue dots at the center of MCE square.

Dot-IGSS: The test was carried out according to Wu *et al*, (1989). The antigen and sera specimens were diluted as required for Dot-ELISA. pH 8.2 TBS containing 10% sheep serum and 1% BSA was used as the blocking solution, while that containing 10% calf serum, was used as the diluting solution. The sheep anti-human IgG was labeled with chloroauric acid by Slot's method (Slot *et al*, 1985) in our laboratory. The working concentration of the labeled IgG was 1:40. When silver nitrate, hydroquinone and citric buffer were added for color development, the appearance of brown-yellow or brown-grey dots at the center of MCE squares.

RESULTS

Positive rates determined by the 3 assays

The results are shown in Table 1.

ELISA revealed the average OD of the 76 negative control sera was 0.1366. As $\overline{X}$+2SD was taken as the cut-off, OD ≥0.2636 indicated positive results.

Comparison of the positive rate of Dot-IGSS with that of ELISA revealed the difference was significant ($X^2 = 6.93$, $p < 0.01$). But differences were insignificant, as Dot-ELISA compared with ELISA and Dot-ELISA compared with Dot-IGSS, where X^2 was 1.28 and 1.57, respectively, and $p > 0.05$ in both cases.

The rates of false positivity found by the 3 assays in the 76 control sera showed no significant differences in comparison between any 2 of them.

The relationship between the reaction intensities in different assays

Fifty-nine of the 65 positive sera were positive in both Dot-IGSS and Dot-ELISA. Rectilineal regression of the reciprocal titers in the 2 assays is shown in Fig 1. Similarly, the relationships between the OD value of ELISA positives and the logarithm of reciprocal titers of Dot-ELISA ($n = 60$) and Dot-IGSS ($n = 64$) positives are shown in Fig 2 and Fig 3, respectively. Significance test of the correlation coefficients (r values) gave $p < 0.01$ in all the 3 analyses.

The results of combined uses of the assays

The number of positive results was 64, 60 and 56 in Dot-IGSS, Dot-ELISA and ELISA, respectively. When the results of Dot-ELISA and ELISA are considered together, 54 sera were positive to both assays but only 3 were not positive in both assays. Hence the rate of positivity in the combined assay will be increased to 95.38% (62/65). Likewise, if Dot-IGSS was used in combination with either Dot-ELISA or ELISA, the rate of positivity would become 100%.

Table 1
Results of anti-*Toxoplasma gondii* antibodies detected by three immunological assays.

Sources of sera	No. cases detected	Dot-IGSS		Dot-ELISA		ELISA	
		No. positive	Positive rate (%)	No. positive	Positive rate (%)	No. positive	Positive rate (%)
Cases infected with *Toxoplasma gondii*	65	64	98.64	60	92.31	56	86.51
Control	76	3	3.95	9	11.84	7	9.21

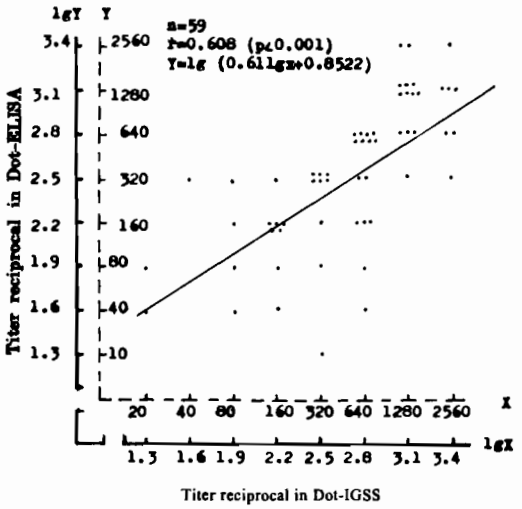

Fig 1–Relationship between the titer of sera in 59 cases infected with *Toxolasma gondii* assessed by Dot-IGSS and Dot-ELISA.

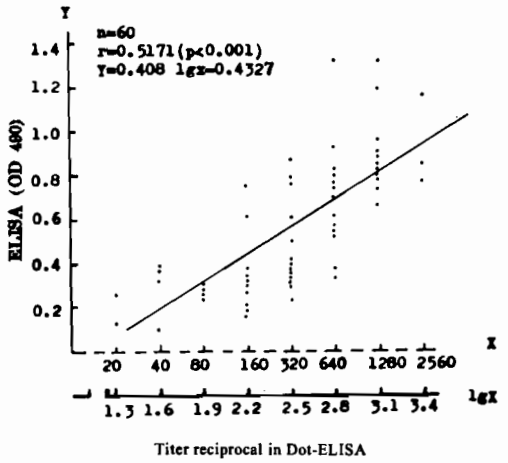

Fig 2–The relationship between the recating intensity of sera of 60 cases infected with *Toxoplasma gondii* assessed by Dot-ELISA and ELISA.

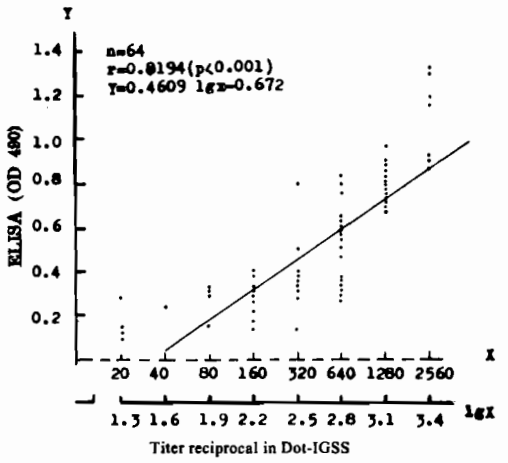

Fig 3–The relationship between the recating intensity of sera of 64 cases infected with *Toxoplasma gondii* assessed by Dot-IGSS and ELISA.

DISCUSSION

Since parasitological examination of toxoplasmosis is very difficult and time-consuming, detection of circulating antigen of *Toxoplasma gondii* in hosts by immunological methods or molecular biological technic was tried to diagnose the disease (Hafid, 1995; Novati 1994). The results thus obtained approach the results of parasitological methods. However, these methods are complicated and require sophisticated facilities, so that they are not practicable in ordinary laboratories. In China, the common serological methods are still in use, but it was advocated that 2 or more methods be used simultaneously to increase the accuracy of anti-*Toxoplasma gondii* detection (Editorial Commit-

tee, 1991). As the serological results are subject to the influence of the experimental conditions in various methods, the results obtained from 2 or more simultaneous assays may be complementary, thus enhancing the sensitivity and accuracy remarkably Jiang and Zhong, 1990).

The present experiment revealed that although the 3 methods were all very sensitive, the positive rates varied considerably, but none of the sera was negative to all the 3 tests. Careful analysis by comparing the results of ELISA and Dot-ELISA showed the positive rates were 86.51% and 92.31%, respectively, and only 3 out of the 65 sera were negative to both assays. Hence the sensitivity of combined ELISA and Dot-ELISA would be increased to 95.38% (62/65). Similarly it was found that the positive rates of combined ELISA and Dot-IGSS, and combined Dot-ELISA and Dot-IGSS would be both 100%, minimizing the possibility of misdiagnosis and enhancing the reliability.

Detection of anti-*Toxoplasma gondii* antibodies of pregnant women has been carried out as a routine in UK (Anonymous, 1990). It was also advocated to take this measure in China (Editorial Committee, 1991). To realize this objective before long, a convenient way is to combine Dot-ELISA with the widely-used ELISA, because they share the essential reagents and can be performed in ordinary laboratories.

Of course, the combined use of Dot-IGSS is a better choice, for Dot-IGSS has higher sensitivity than ELISA and Dot-ELISA as has been used in the immunological diagnosis of some helminthiasis (Wu *et al*, 1989, 1991; Liu, *et al*, 1994, 1996). It is believed that the combined use of Dot-IGSS with ELISA and/or Dot-ELISA will become a reliable immunological method to diagnosis toxoplas-mosis and can be popularized once a Dot-IGSS test kit containing colloidal gold-labeled anti-human IgG is commercially available.

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