Short Paper

Standardization and comparison of Dot-ELISA with IFA test for diagnosis of human toxoplasmosis

Nourollahi Fard, S. R.1; Shad-Del, F.2*; Hosseini, S. M. H.3 and Razavi Dinani, S. M.4

1Department of Parasitology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
2Department of Parasitology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran
3Razi Vaccine and Serum Research Institute, Shiraz, Iran
4Department of Pathobiology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran

*Correspondence: F. Shad-Del, Department of Parasitology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran. E-mail: fazlolah162002@yahoo.com

Summary

Dot-ELISA assay is a solid phase diagnostic method for detection of antigen or antibody that is used widely for diagnosis of protozoan and metazoan diseases of human and animals. To evaluate this method in diagnosis of human toxoplasmosis, the test was standardized, using golden positive and negative serum samples. Then 215 human serum samples were evaluated for IgG and IgM against Toxoplasma gondii by Indirect Immuno-Fluorescent Antibody (IFA) and Dot-ELISA. Using statistic program, Epi info 6.0 showed these tests have a good agreement in diagnosis of toxoplasmosis with Kappa = 0.8607 for IgG and Kappa = 0.8865 for IgM antibodies (P<0.05). This study and the works carried out by the other scientists indicate that the Dot-ELISA test is rapid, simple, cost effective, does not need expensive equipment and has a good sensitivity and specificity. But, as the results are expressed qualitatively, therefore, it is not possible to use for antibody titer determination. Yet, it is quite useful for screening test especially in the field and where there are no well-equipped laboratories.

Key words: Dot-ELISA, Indirect Immuno-Fluorescent Antibody (IFA), Toxoplasmosis

Introduction

In spite of the advances made in diagnosis of bacterial, viral and protozoan diseases, diagnostic methods have to be renewed to be more rapid, sensitive and specific. During the past few years, there has been an increased interest in the diagnosis of parasitic diseases using techniques, which are rapid, simple and inexpensive. Old serological procedures such as indirect haemagglutination (Jacobs and Lunde, 1957), complement fixation test (Kent and Fife, 1963), counter immuno-electrophoresis (Hutchison et al., 1970; Gentilini and Pinion, 1972) and immuno-fluorescence (Duxbury and Sadun, 1964) are tedious and difficult to standardize, conduct and interpret. Also the reagents are consumptive and require highly trained technicians as well as expensive instruments such as fluorescent microscope.

Toxoplasma gondii is a coccidian parasite of the cat and its infection may lead to major public health problems (Hutchison et al., 1970; Gentilini and Pinion, 1972). Though most human infections are subclinical and even clinical infections are rarely fatal, however, in pregnant women this organism may cross the placenta and infect the fetus with serious consequence (McColm and Hutchison, 1981). Since the diagnosis of clinical toxoplasmosis is often difficult, serological methods have been employed in aid of diagnosis. The most used assay i.e., Indirect Immuno-Fluorescent Antibody (IFA) test, requires expensive fluorescent microscope (Duxbury and Sadun, 1964) and trained technicians, so this research was
planned, in parallel with the IFA test, to standardize the Dot-ELISA technique, which is simple to perform and doesn’t need expensive equipment to detect IgG and IgM specific antibodies against *Toxoplasma gondii*.

**Materials and Methods**

*Toxoplasma gondii* tachyzoites (RH. Strain) were kindly supplied through Dr. Asmar and Dr. Piaza from Department of Parasitology, Pasteur Institute, Tehran, Iran. 

*Toxoplasma* tachyzoites were collected from peritoneal cavities of Balb-c mice inoculated 3 days previously and separated from peritoneal exudate cell by centrifugation at 2000 g for 15 min. The tachyzoites were adjusted to 50 organisms per high power field of microscope. Twenty µl of this suspension were loaded on ringed area on glass slides (Behring Werk A.G.), dried up and stored at -20°C, until required.

Antigen was prepared for Dot-ELISA by washing parasites as above. Then the fluid was centrifuged and the parasites were suspended in distilled water and ruptured with the sonicator set at 60 cycles for the periods of 30 seconds. After checking the suspension under microscope and being sure that no more intact parasites remained, it was centrifuged and the supernatant was decanted. This was adjusted into 2 mg/ml, aliquots, and were stored at -20°C until used.

**Protein determination**

Protein content of antigen was determined using Lowery technique, then by adding D.W. the final protein concentration was adjusted to 2 mg/ml.

**Sera**

Serum samples were prepared from 215 blood samples obtained from patients by Dr. Azad in Immunology Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran. The sera were divided into couple aliquots and stored at -20°C, until used.

**Indirect fluorescent antibody test**

The sera were tested in the dilution of 1:64 using standard technique for IFA test. For this study, FITC conjugated Rabbit Anti-Human Immunoglobulins (DAKO, Denmark) was used in a dilution of 1:1500. All samples were examined under Zeiss fluorescent microscope. Fluorescent staining was considered negative if the tachyzoites fluoresced only at their anterior end. The titers of 1:64 and above were considered as positive and the sera with titers of up to 1:1024 and those of less than 1:16 were considered as golden positive and absolute negative sera, respectively for standardization of Dot-ELISA assay.

**Dot-ELISA**

Nitrocellulose strips, 0.45 µm Millipore were put in distilled water for 2 min, then after drying at 37°C the strips were spotted with 1 µl of antigen, using Hamilton syringe after determining that 1 µl of antigen gives good result (fig. 1). After drying at 37°C for 15 min, antigen uncoated sites on the nitrocellulose strip were blocked with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS), being shaken for one hour at room temperature. Then the strips were again washed three times with PBS containing 0.05% Tween 20 for 10 min. with constant shaking. Nitrocellulose strips with control positive, control negative and test sera were incubated at 37°C for 30 min, with constant shaking. The dilution of 1:400 was determined as the best (fig. 2). Then the strips were washed three times as above and a second antibody, peroxidase conjugated Rabbit Anti-Human Igs. (DAKO, Denmark) was used at dilution 1:8000 and incubated at 37°C for 30 min, with constant shaking. After washing as above, the strips were exposed to a liquid 3, 3’-diaminobenzidine (DAB) containing H2O2 at pH 7.5 (DAKO, Denmark), as substrate-chromogen system for 4 min, with gentle shaking and the colour development was stopped with tap water. Positive reaction was determined by appearance of clearly defined brown dots at the sites of antigen (fig. 3). The reaction was considered negative, when the nitrocellulose strips had no coloured dots.
Table 1: The results of Dot-ELISA and IFA for detection IgG and IgM antibodies against *Toxoplasma gondii*

<table>
<thead>
<tr>
<th>Test</th>
<th>IFA-IgG</th>
<th>Dot-ELISA-IgG</th>
<th>IFA-IgM</th>
<th>Dot-ELISA-IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>106 (49.3%)</td>
<td>121 (56.2%)</td>
<td>11 (5.11%)</td>
<td>15 (6.97%)</td>
</tr>
<tr>
<td>Negative</td>
<td>109 (50.7%)</td>
<td>94 (43.8%)</td>
<td>204 (94.88)</td>
<td>200 (93.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>215</td>
<td>215</td>
<td>215</td>
</tr>
</tbody>
</table>

Fig. 1: Comparison of different amounts of antigen (μg per ml) used for standardization of Dot-ELISA
Fig. 2: Comparison of different dilutions of test sera and peroxidase conjugated Rabbit Anti-Human Igs in Dot-ELISA

Fig. 3: Positive and negative reactions in Dot-ELISA
Results

In this study the Dot-ELISA was standardized for detection of antibodies against the *Toxoplasma gondii* in human sera and compared with IFA test. Out of 215 serum samples tested by IFA 106 samples (49.3%) were positive for IgG, at the titer of 1:64 and 121 samples (56.2%) were positive by Dot-ELISA (Table 1).

The sera also were tested by both IFA and Dot-ELISA assay for detection of IgM antibodies. In IFA test 11 samples (5.11%) and in Dot-ELISA test 15 samples (6.97%) were positive for IgM antibodies (Table 1).

Discussion

The Dot-ELISA has been employed by some researchers for detection of *Toxoplasma* antigen or antibody. Yamamoto *et al.*, (1998) used this method for detection of IgG, IgM and IgA antibodies against excreted-secreted (E/S) antigens of *Toxoplasma gondii*. In their study E/S antigens from peritoneal exudates of infected mice were precipitated with 40% ammonium sulphate, then were used in immunoblot assay and Dot-ELISA. Liu *et al.*, (1997) used the combination of Dot-ELISA and Dot-Immunogold silver staining (Dot-IGSS) simultaneously to detect the specific IgG against *Toxoplasma*. In their study, no statistically significant differences were observed between ELISA and Dot-ELISA or between Dot-ELISA and Dot-IGSS (P>0.01). Elsaid *et al.*, (1995) used Dot-ELISA for diagnosis of human toxoplasmosis. Out of 538 serum samples tested by IFA-IgG as reference test, 183 (34%) were positive at dilution of 1:16 and 192 (36%) were positive for Dot-ELISA at dilution of 1:256. For Dot-ELISA co-positivity was 0.94, co-negativity 0.94 and concordance 0.88 in relation to IFA-IgG. The results suggest the usefulness of Dot-ELISA for the serodiagnosis of human toxoplasmosis.

Youssef *et al.*, (1992) evaluated the efficacy of Indirect Haem- Agglutination (IHA), IFA and Dot-ELISA in serodiagnosis of toxoplasmosis in complicated pregnancies. Investigation on 72 pregnant woman having congenital anomalies, stillbirth and repeated abortion at first and second trimesters showed *Toxoplasma* antibodies with high-titer by IFAT and Dot-ELISA. Analysis of the results showed a positive correlation between IFA and Dot-ELISA but not with IHAT. Pappas *et al.*, (1986) compared Dot-ELISA and ELISA with IFA test for detection of IgG and IgM specific antibodies to human toxoplasmosis. The results showed that Dot-ELISA correlated with IFA test (correlation coefficient = 0.895) and ELISA correlated slightly higher with IFA test (correlation coefficient = 0.910) for detection of IgG antibodies to *Toxoplasma gondii*.

The present study, using statistic program Epi info 6.0 showed these tests have a good agreement in diagnosis of toxoplasmosis with Kappa = 0.8607 for IgG (Agreement rate = 93.02%) and Kappa = 0.8865 for IgM antibodies (Agreement rate = 98.1%) (P<0.05). Correlation coefficient was 0.869 for IgG and 0.847 for IgM antibodies (P<0.05).

The IFA technique is probably the most used serological method for diagnosis of toxoplasmosis. However, this method needs fluorescent microscope, which is expensive and restricts its use mainly to central diagnostic laboratories. It also requires trained technicians and is somewhat subjective. The main advantage of this technique is its simplicity and also this technique is rapid, easy to perform, cost effective and field portable. The Dot-ELISA has been used extensively in the detection of human and veterinary protozoan and metazoan parasitic diseases (Duxbury and Sadun, 1964; Gentilini and Pinion, 1972; De Hubsch *et al.*, 1988; Maria *et al.*, 1990; Jacqueline, 1999). It does not require special equipment and can be used as a qualitative test to screen large number of samples.

Acknowledgments

Thanks are due to Dr. Asmar and Dr. Piaza for furnishing the strain and Dr. Azad for supplying the test sera.

References

1- De Hubsch, RM; Chiechie, N; Comach, G; Rangel Aldo, R; Gusmao, RD (1988). Immunoenzyme assay using micro Dot on nitrocellulose (Dot-ELISA) in the diagnosis of