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Comparison of Horse Derived *Toxoplasma gondii* as Antigen with a Commercial Available ELISA Kit for the Detection of IgG Antibodies in Human Sera

R.M. Shaapan and Nawal A. Hassanain

Department of Zoonotic Diseases, Veterinary Research Division,
National Research Center, Post Box 12622, El-Tahrir Street, Dokki, Giza, Egypt

Abstract: Commercial kits for the detection of *Toxoplasma* IgM and IgG antibodies are increasingly being used in laboratories throughout the world. A potential problem with many of these kits is the unacceptably high numbers of false-positive and false-negative results. Therefore, further research concerning the antigen used is required to improve the quality of the assay being used with lower cost. An attempt to isolate locally horse *T. gondii* strain and prepare an antigen was carried out. We compared the sensitivity of standard ELISA assay using this antigen and a commercial kit for detecting IgG antibodies in 200 human serum samples collected from suspected cases of toxoplasmosis. Results showed that ELISA assay developed in our laboratory using the same serum dilution (1:51) and incubation time (1/2 hr) of the commercial kit gave comparable results (46% positive) with the commercial kit (45% positive). On the other hand, ELISA assay developed in our laboratory using higher serum dilution (1:100) and longer incubation time (1 hr) displayed higher results (49% positive). So, this study can suggest that the standard IgG ELISA assay with local *T. gondii* strain antigen and with these conditions is more sensitive than the commercial ELISA kit.

Key words: Toxoplasmosis · man · ELISA · IgG · commercial kit

INTRODUCTION

Toxoplasmosis is a zoonotic disease and is transmitted to humans mainly by ingesting food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts [1, 2].

Most infections produce minimal, if any, symptoms and have no ill effect on the patient (if symptoms do occur, they may be flu-like and include fatigue, muscle aches, malaise, fever, etc). However, if a woman who has not previously been infected with *T. gondii* becomes infected just before or in pregnancy, may produce abortion, with fetal neurological disorders [3].

Serological tests constitute the cornerstone in the diagnosis of infection with *T. gondii*. Sabin-Feldman dye test [4], indirect hemagglutination assay [5] and the indirect immunofluorescence assay [6] are among the earliest and the best characterized serological tests for diagnosis of Toxoplasmosis. However, viable *T. gondii* tachyzoites are required for the Sabin-Feldman dye test and a considerable sample manipulation is necessary for IHA (particularly if nonspecific agglutinins are present, since these samples require agglutinins adsorption and retesting). Various enzyme immunoassays (EIAs) have also been evaluated for their ability to detect anti *T. gondii* antibodies in serum [6-8].

A potential problem of unacceptably high numbers of false-positive and false-negative results was obtained by using the commercial kits for the detection of toxoplasma IgM and IgG antibodies in laboratories [9, 10]. The specific strain of *T. gondii* used to produce the antigen may not match the strain the patient has.

Therefore, further research concerning the antigen used is important to improve the quality of the assay being used with lower cost. So the aim of this study is to adjust *T. gondii* antigen from locally isolated strain followed by using it in an ELISA procedure for detecting IgG antibodies in human sera and comparing the results with those of a commercially available ELISA kit.

MATERIALS AND METHODS

Serum samples: A total number of 200 human serum samples routinely examined from suspected cases of toxoplasmosis were obtained from specimens

Corresponding Author: Dr. Raafat M. Shaapan, Department of Zoonotic Diseases, Veterinary Research Division, National Research Center, Post Box 12622, El-Tahrir Street, Dokki, Giza, Egypt

that had been submitted to the clinic of the Department of Parasitology at Faculty of Medicine, Ein-Shams University; Cairo, Egypt. The patients were of different ages, sexes and showed different clinical signs in the form of fever, arthritis, reproductive manifestations, eye disorders and abortion. Each sample was labeled in serial number and stored at -20°C until use. Only one sample from each patient was accepted for testing by ELISA using both a commercial kit and locally isolated antigen.

ELISA kit: Two package of a commercially avialible ELISA kit (Clinotech®Dignostics Toxoplasma IgG ELISA) were brought and stored at 2-8°C until used for detection of anti *T. gondii* IgG antibodies according to the instructions of the manfactur.

Assay procedure: The clinotech Toxo IgG ELISA test is designed for the qualitative detection of IgG antibodies to *T. gondii* in human sera. The wells are coated with antigens. Diluted patient serum and control ones are incubated in the wells. The Toxo IgG specific antibodies if present, bind to the solid phase antigens. All unbound antibodies are washed off. Horse Radish Peroxidase (HRP) Conjugated anti human IgG is added. The unbound HRP conjugate is washed off. Upon addition of TMB substrate, the bound enzyme generates color. The intensity of the color is directly proportional to the concentration of anti Toxo IgG in the samples. 1:51 dilution of each test sample (negative control, positive control and calibrator are pre-diluted) was prepared. Color reaction of each test sample was read at 450 nm.

Interpretation of results: OD ratio for each specimen was obtained by dividing its OD value by calibrator OD as follows: Specimen OD ratio = Specimen OD/ Ccalibrator OD

OD ratio is interoperated as follows:

- An OD ratio = 0.90 indicates no detected antibody to *T. gondii.* A negative result indicates no current or previous infection with *T. gondii.*
- An OD ratio = 1.00 is positive for IgG antibody to *T. gondii.* A positive value indicates a current or previous infection with *T. gondii.*
- Specimens with OD ratio in equivocal range (0.91- 0.99) should be retested.

ELISA using locally isolated antigen

Antigen preparation: The local strain *T. gondii* tachyzoites was isolated from tissues of slaughtered horses at Giza-Zoo abattoir, Egypt, when bioassayed in mice and cats [11]. These tachyzoites were successfully maintained in the laboratory [12] and used for the preparation of *T. gondii* antigen for ELISA [13], Briefly, tachyzoites were repeatedly freezed and thawed to rupture the parasite wall, sonicated and centrifuged at 12,000 rpm for 45 min at 4°C. The supernatant was collected and its protein content was determined [14].

Standard enzyme linked immunosorbent assay procedure: The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration and test procedure was carried out [15]. In the present study, the optimum conditions were 40µg protein/ml coating buffer antigen concentration, 1:100 serum dilutions, 1: 1000 anti-human IgG HRP conjugate (Sigma Co.) and 3 mg OPD dissolved in 10 ml 0.2M substrate buffer containing 0.03% H_2O_2 as substrate. The absorbance of the colored reaction was read within 30 min using a titertek multiskan ELISA reader at Optical Density (OD) of 490 nm. All incubation steps were carried out at 37°C in a moist chamber.

Modified enzyme linked immunosorbent assay procedure: The previously mentioned standard ELISA procedure was repeated using the same locally *T. gondii* antigen with a slight modification as we reduce the time of incubation to become half an hour instead of one hour but duplicate the serum dilution (1:51) to resembling the incubation time and sera dilutions used in ELISA assay kit.

Reading and interpretation of results: The cut off values of optical densities were calculated [16]. Cut off value = mean OD of –ve control + 3 standard deviation (SD).

RESULTS

The results are shown in Table 1, Fig. 1-4: Out of 200 tested human sera, 90 (45%), 98 (49%) and 92 (46%) were

Table 1: Results of the different ELISA assays

	No of examined	Positive reactors		Negative reactors	
Assay	sera	No	%	No	%
ELISA using commercial kits	200	90	45	110	55
ELISA using local Ag	200	98	49	102	51
ELISA using local Ag with 1/2 incubation time	200	92	46	108	54

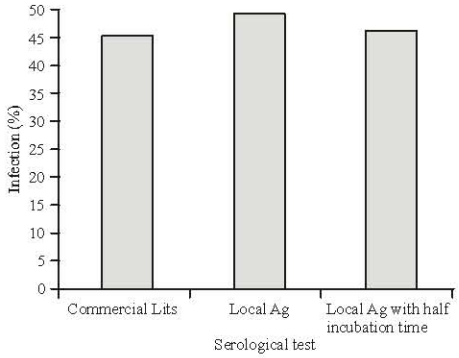

Fig. 1: Toxoplasmosis in humans by different of ELISA assays

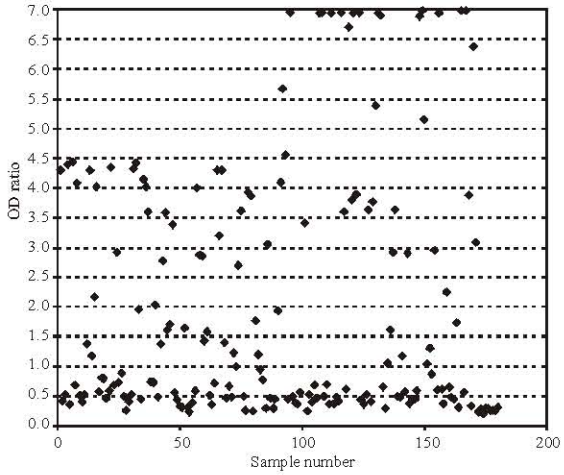

Fig. 2: Scatter graph representing diagnosis of Toxoplasmosis using ELISA Kit

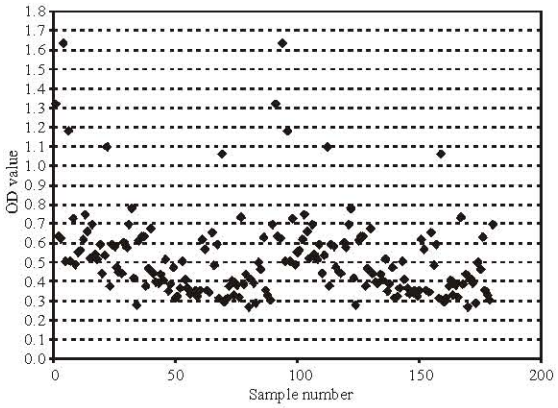

Fig. 3: Scatter graph representing diagnosis of Toxoplasmosis using local antigen

Toxoplasma gondii seropositive by ELISA assay using commercial kits, local antigen and local antigen with 1/2 time of incubation respectively.

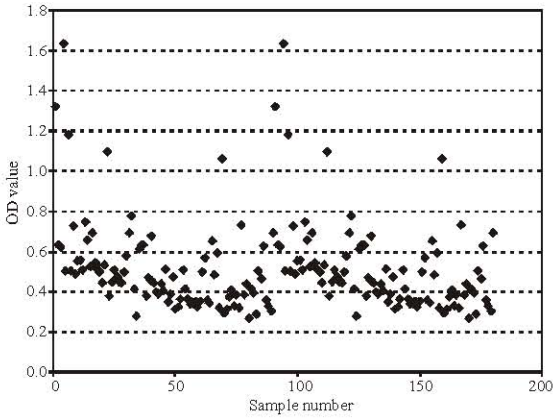

Fig. 4: Scatter graph representing diagnosis of Toxoplasmosis using local antigen using half time of incubation

DISCUSSION

Toxoplasmosis is a protozoan disease that shows varying clinical manifestations. Determination of its incidence in various risk groups in the society and establishment of these risk groups play a significant role in taking the necessary precautions against this disease.

In the present study, high positive anti-*Toxoplasma* IgG was detected in human sera by ELISA using commercial kits (45%), local antigen (49%,) and local antigen with 1/2 time of incubation (46%). These results agreed with those of Abdel-Hafez *et al.*, El-Hawey *et al.* and Hassan *et al.* [17-19] who also reported a high anti-*Toxoplasma* IgG prevalence in human sera ranged from 45 to 55%. While the results was nearly varied from that results of the studies carried out in New York, London and Paris which reported positive anti toxoplasmosis antibodies rate of 32, 22 and 84%, respectively [20].

In Egypt studies on the prevalence of toxoplasmosis in Qualyobia Governorate, reported seropositivity to IgG antibodies 36.4, 59.2 and 57.9%; IgM antibodies 27.3, 7.4 and 10.5% in complicated gestation, uncomplicated gestation and random population, respectively and IgG antibodies 55.5 and 40% in normal full term babies and abnormal babies [21]. While *Toxoplasma* antigen (TAg-ELISA) in human serum samples and the target antigen in 88% sera of acutely infected women detected [22].

ELISA results showed an excellent agreement between the results obtained with the commercial kit (45% positive) and those with a comparison method, the

standard IgG ELISA assay developed in our laboratory (46% positive), with the same serum dilution (1:51) and incubation time (1/2 hr). So, we suggest that the two assays may have the same sensitivity. On the other hand, the standard IgG ELISA assay developed in our laboratory with higher serum dilution (1:100) and longer incubation time (1 hr) gave higher results (49% positive), than those of the commercial kit (45% positive). The differences in serum dilution and incubation times between the two assays, however, prevent them from being run simultaneously. So, we can suggest that the IgG ELISA assay standard developed may be more sensitive than the commercial ELISA kit.

CONCLUSION

It was concluded that the serological tests for toxoplasmosis using ELISA with antigen prepared from locally horse isolated *T. gondii* strain, serum dilution (1:100) and incubation time (1 hr) is more sensitive and cheaper than the commercial kit. Also this study has provided detailed, objective information which should be of use to local laboratories and placed the commercial kits in relevant groups. It is up to local laboratories with a consideration of their local circumstances, to select the most appropriate assays.

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