

Comparison of the *Toxoplasma gondii* mice and cell culture derived antigens in ELISA assay

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Abstract. Toxoplasmosis is an infectious disease caused by the coccidian parasite *Toxoplasma gondii*. Diagnosis is based on serological methods with detection of specific IgG and IgM antibodies. The present study was performed to compare the sensitivity and specificity of soluble antigen of *T. gondii*, RH strain obtained from mice and cell culture in ELISA method. Tachyzoites of *T. gondii*, RH strain that inoculated in mice peritoneum were collected. At the same time, tachyzoites were harvested from HeLa cell culture that infected with the parasite. Soluble antigen was prepared and ELISA method performed on 100 serum samples that were collected from different laboratories in Tehran, Iran. Commercial Trinity kit was used as gold standard. The sensitivity and specificity of *T.gondii* soluble antigen were higher in antigens that obtained from cell culture in comparison with mice peritoneum. *T. gondii* cell culture derived antigen has high sensitivity and specificity in ELISA test.

INTRODUCTION

Toxoplasma gondii the intracellular protozoan parasite has medical and veterinary importance. *T. gondii* infection is severe and life threatening in immunocompromised patients and in congenital form (Dubey, 2010).

Diagnosis is based on pathological, serological and molecular methods. Among these methods serological tests have high levels of sensitivity and specificity (Pereira-Chioccola *et al.*, 2009). The ELISA is one of these serological methods that has been modified for detection of both Ig G and IgM antibodies and for differentiation between acute from chronic toxoplasmosis (Naot & Reminton, 1980; Rahbari *et al.*, 2012; Ali-Heydari *et al.*, 2013).

Tachyzoites of *T. gondii*, RH strain that have been used for detection of toxoplasmosis in ELISA method, are inoculated in mice peritoneum, routinely.

In this way antigens have impurities such as proteins and cells of mice that these impurities could affect the results of serological tests (Costa-Silva *et al.*, 2012; Costa-Silva *et al.*, 2008). The in vitro culture of *T. gondii* tachyzoites and production of large amount of pure antigen has been established for years.

The present study was performed to compare the sensitivity and specificity of soluble antigens of *T. gondii* tachyzoites from mice peritoneum and cell culture for detection of toxoplasmosis in ELISA method.

MATERIAL AND METHOD

Tachyzoites of *T. gondii*, RH strain were collected from peritoneal cavity of Balb/c mice that were infected three days before. Tachyzoites were washed with PBS (phosphate buffer saline), (pH=7.2) for 3 times, sonicated and centrifugated at 14000

rpm for 1 hour in 4°C. The supernatant was collected and protein density was measured by Bradford method (Bradford, 1976).

Cell culture

HeLa cells were grown in 25 cm² culture flasks (Nunc, Denmark) in 10 ml of culture medium containing; Dulbeccos, modified eagle (DMEM/KB cell, Iran) supplemented with 10% inactivated fetal calf serum (FCS, Bovogen, Australia), 10 mM hepes and 1% penicillin, streptomycin (Biowest, France) and incubated at 37°C in 5% CO₂ atmosphere.

After forming of confluent monolayer, the cells were infected with tachyzoites at a 1:1 ratio. After 4 hours the media was removed and culture medium containing 5% FCS was added, 1 day later, the FCS free medium was added and after 3 day the tachyzoites were collected, washed and soluble antigen was prepared as described.

ELISA

Microtiter 96 well plates (Nunc, Denmark) was coated with 100µl of soluble antigen of *T. gondii*, RH strain from mice peritoneum and cell culture respectively and stored at -20°C until use. Plates were incubated overnight at 4°C. Sera were diluted 1:200 in PBST (PBS plus 0.05% tween 20). After washing 100µl of diluted serum was added, after incubation for 45 minutes and 3 times of washing, anti-human IgG conjugated with HRP (horse radish peroxidase, DAKO, Denmark) in dilution of 1:500 was added. After incubation and washing the chromogenic substrate orthophenylen-diamidine (OPD) (Merck, Germany) was added and finally the reaction was stopped by adding of 20% sulfuric acid. The results were recorded by an automated ELISA- readerer (BIOTEC, LX800, USA). The amounts of antigen and the dilutions of sera and anti- human IgG were optimized by checker board method. In each procedure 30 negative sera was tested and the cutoff was determined as the mean plus two times of the standard deviation of the absorbance readings obtained for the negative samples ($X \pm 2SD$). The optical densities more and less than cut off were considered as positive and negative respectively.

In this study Trinity kit (Trinity, USA) was used as gold standard. All the samples were checked for diagnosis of anti- *T. gondii* IgG antibody by this kit and the parameters of sensitivity, specificity, validity, concordance, positive predictive value (PPV) and negative predictive value (NPV) for both antigens derived from mice peritoneum and cell culture were estimated (Selseleh *et al.*, 2012).

RESULTS

The anti- *T. gondii* IgG antibody was checked in 100 human serum samples by the soluble antigens of *T. gondii*, RH strain tachyzoites derived from HeLa cell culture and mice peritoneal cavity in ELISA method (Table 1).

Table 1. The results of ELISA test with soluble antigens of tachyzoites of *T. gondii*, RH strain from HeLa cell culture and mice peritoneal cavity

(%)	<i>T. gondii</i> soluble antigen from HeLa cells	<i>T. gondii</i> soluble antigen from peritoneal cavity
Specificity	93	85.4
Sensitivity	91.5	80.8
Validity	92.2	83.2
Concordance	82	76
P.P.V*	91.5	80.8
N.P.V**	93	85.5

* positive predictive value

** negative predictive value

DISCUSSION

Toxoplasmosis, the widespread infection is caused by the protozoan parasite, *Toxoplasma gondii*. Human toxoplasmosis could be diagnosed by different methods that among these methods, serological assays have major value for detection of specific antibodies (Dubey, 2010).

The lysate of *T. gondii*, RH strain tachyzoites that has been passaged in mice peritoneum is the most common source of

antigen for immunological test (Costa-Silva, *et al.*, 2008; Lee *et al.*, 2008).

According to ethical issues, infra-structural deficiencies and maintenance costs cell culture systems are preferred in the most laboratories and research centers than animal models (Chatterton *et al.*, 2002; Ashburn *et al.*, 2000; Evans *et al.*, 1999).

Fresh viable tachyzoites could provide by continuous cell cultures in different cell lines which are easy to maintain and inexpensive (Evans *et al.*, 1999).

Ashborn *et al.* used cell culture derived tachyzoites for dye test method and concluded that these tachyzoites could be used routinely with more cost effectiveness than animal culture (Ashborn *et al.*, 2000). Evan *et al.* compared three cellular classes of HeLa, Vero and LLC and the number of separated tachyzoites of HeLa cell culture was reported to be higher (Evans *et al.*, 1999).

In the present study soluble antigen of tachyzoites of *T. gondii*, RH strain provided from mice inoculation and HeLa cells culture were used in ELISA method for detection of *T. gondii* infection in human serum. The results of ELISA test were checked by commercial Trinity kit as gold standard. According to the results obtained here, the cell culture derived antigen showed high sensitivity and specificity in comparison with antigens collected from mice peritoneum. Other parameters such as validity, concordance, PPV and NPV were higher in cell culture derived antigen. The tachyzoite load was higher and the overall cost was lower in cell culture system than mice inoculation.

CONCLUSION

Establishment of *T. gondii* cell culture system in a laboratory, in addition to improve the results of diagnostic tests is more ethical than animal models with low costs and more efficacies in large scale research projects. In parallel to cell culture system, animal passages recommended to minimize the

reduction of parasite virulence in research centers.

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There is no conflict of interests.

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