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**Original Article**

# Detection of *Toxoplasma gondii* Antigens in Sera and Urine of Experimentally Infected Mice by Capture ELISA

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## Abstract

**Background:** *Toxoplasma gondii* is an apicomplexan parasite which infects a broad range of hosts. The classical diagnosis of toxoplasmosis relies on serological methods. Detection of parasite or its components could be useful tool for early diagnosis of the infection.

**Methods:** Fifty mice were infected by the intraperitoneal route with 5000 tachyzoites of *T. gondii* RH strain. Five of them sacrificed every day from day 1 up to day 7 post infection. Sera and urine of mice were tested by capture ELISA.

**Results:** *T.gondii* antigens were detected from 3rd and 2nd day in serum and urine, respectively, after infection until their death on day 7.

**Conclusion:** Antigenemia detection of antigens of parasite was possible in a short period of acute infection with *T. gondii* by capture ELISA.

**Keywords:** Toxoplasma gondii**,** *Antigenemia, Capture ELISA*

## Introduction

*Toxoplasma gondii* is an obligate protozoan parasite, which infects a broad range of warm-blooded animals. Toxoplasmosis has little clinical manifestation in immunocompetent individuals. However, it can be serious or fatal in infected children during intra-uterine development and immunocompromised patients (1). Primary acquired infection during pregnancy may cause miscarriage, permanent neurological damage, premature birth, and visual impairment (2-4). Toxoplasmic encephalitis is seen in *Toxoplasma*-infected immunocompromised patients frequently (5-8). Early diagnosis of active toxoplasmosis in pregnant women can prevent severe congenital infection in the developing fetus (9, 10).

Thus, there have been many studies to innovate a more reliable and efficient technique for rapid diagnosis of this parasitic infection (6, 11-13).

The classical diagnosis of toxoplasmosis relies on serological methods and detecting of specific immunoglobulin antibodies. However, these methods have poor efficiency, especially in neonates and immunodeficient patients (1, 14). Several reports have emphasized on detecting *T.gondii* antigens in urine for diagnosis of acute stage of toxoplasmosis (15-19).

The aim of the present study was to detect *T. gondii* antigens in sera and urine of acutely infected mice by capture ELISA.

## Materials and Methods

Antigens were prepared from tachyzoites of *T.gondii* RH strain. Tachyzoites were collected from the peritoneal exudates of 22-24 gr mice infected 3-4 days earlier in sterile PBS, pH 7.2. The organisms were centrifuged at 2000g for 20 min, washed three times in PBS and disrupted in the cold by sonication. Lysed cells were centrifuged at 12000g for 1 hour at 4 $^{o}$C. The supernatant was collected and used as the soluble *T.gondii* antigen. Protein determination

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was performed using the method of Bradford (20). In order to obtain polyclonal antiserum, two rabbits were immunized with soluble *T.gondii* antigens, RH strain, blended with Freud's adjuvant in three times. Polyclonal antibody was isolated from their sera with ammonium sulfate precipitation and Ion-exchange column chromatography. Polyclonal antibodies were tested by SDS-PAGE and immunoblotting to confirm purification and specification. Then polyclonal antibodies were conjugated with HRP (Horse Radish Peroxidase) enzyme by means of periodate method, according to Kawaoi and Nakane (21). This conjugate was stored frozen until use (21, 22).

Fifty male Balb/c mice weighing 17-20 gr were inoculated by the intraperitoneal route with $5X10^3$ tachyzoites of *T.gondii* RH strain. Five of them were anesthetized by ether for ethical issues, and then were sacrificed everyday from day 1 up to day 7 post infection. Sera and pooled urine were collected from 24 hours at time intervals up to six days post infection. All reminding mice (twenty mice) died on day 7 post infection. Whole blood was removed from each animal by cardiac puncture. Sera and pooled urine were centrifuged, and supernatant was kept at -20 $^oC$ until use. Sera and urine from uninfected mice (inoculated with sterile saline) were used as negative controls, too. Ethical Committee of the university approved the study as for observing the animal rights.

Capture ELISA (1) was performed on sera and pooled urine of mice. Multilevel plates (Nunc) were absorbed with polyclonal rabbit antiserum to *T.gondii* at a concentration of 30 $\mu g/ml$ in coating buffer (PBS, pH 7.2). After overnight incubation at 4 $^oC$ and washing, the samples (sera or urine) were added to each well and held for 1 hour at 37 $^oC$.The plates were washed three times with 0.05% Tween 20 in PBS (PBST).Anti-rabbit IgG horseradish peroxidase-conjugated antibodies was diluted 1:25 in PBT, added to each well and held for 1 hour at 37 $^oC$. After washing, the chromogenic substrate ortho-phenylen-diamidine (sigma-Aldrich) was added to each well. After incubating for 20 min the enzymatic activity was revealed. The reaction was stopped by addition of sulfuric acid 20%. Absorbance (Optical density) was recorded at 492nm, as detected with an automated ELISA reader. The cutoff was determined as the mean plus two times the standard deviation of the absorbance readings obtained for the negative samples(1).

## Results

High titers of polyclonal antiserum were found in sera of two immunized rabbits with soluble *T. gondii*, RH strain antigens. Actually one booster injection produced acceptable response (Fig.1).

Polyclonal antibodies were purified from rabbit sera with ammonium sulfate precipitation and ion-exchange column chromatography. One definite protein band with molecular weights of 150 kDs was detected in isolated polyclonal antibody by SDS-PAGE (Fig. 2)

The specificity of isolated polyclonal antibody was determined by immunoblotting.

The parasitic components were detected in mice sera from day 3 after inoculation of *T.gondii* tachyzoites by capture ELISA. *T.gondii* antigens were detected in 40% of mice sera in three days post infection. In addition, antigenemia was confirmed in 100% of mice sera from day 4 up to their death on day 7. No positive reactions were demonstrated in sera of control mice. The results from mice urine, in comparison with those obtained from sera, was 1 day earlier. Actually, *T.gondii* antigens were detected in pooled urine of mice from day 2 after inoculation of tachyzoites.

No evidence of positive reaction was detected in urine of control mice. Although, peritoneal fluid of both test and control group were examined every day for presence or absence of *T.gondii* tachyzoites.

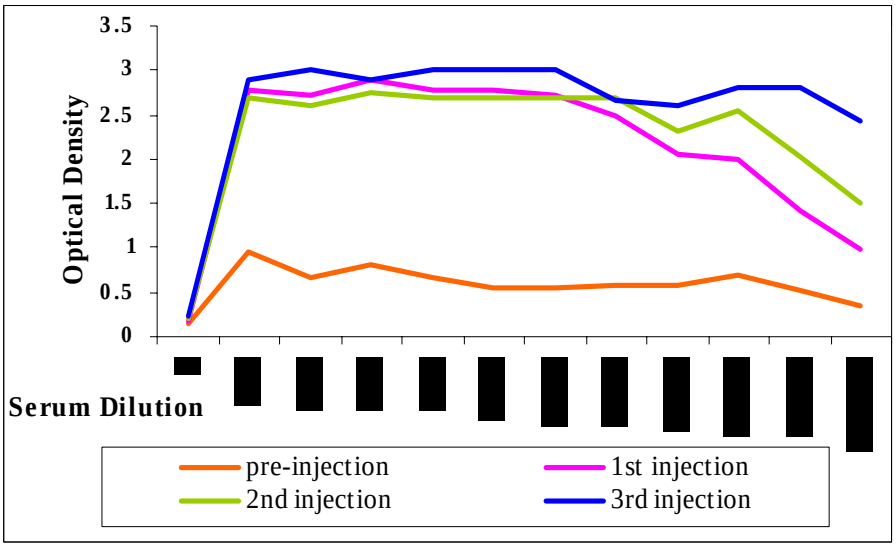


**Fig. 1:** Trends of antibody rising after injection of *T.gondii* antigen and Freunds adjuvant in rabbit by ELISA

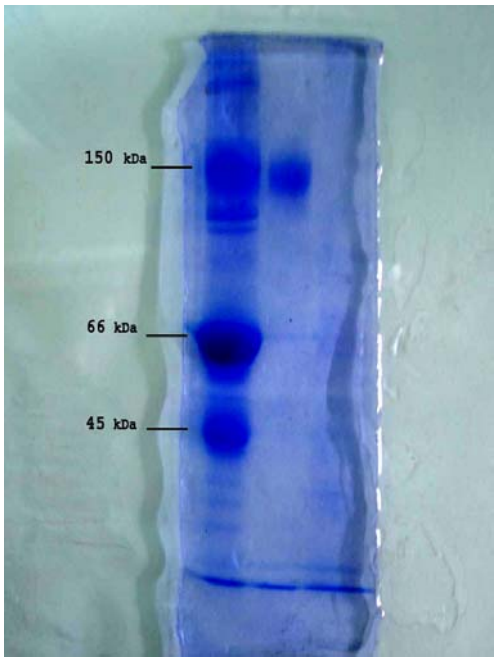


**Fig. 2:** Isolation of IgG from Immunized rabbit sera with *T.gondii* by saturated ammonium sulfate and Ion-exchange chromatography. Left; marker (IgG,Albumin,Ovaalbumin), Right; Isolated IgG.

## Discussion

The diagnosis of recently acquired *T.gondii* infection is usually based on the detection of specific IgM antibodies or seroconversion (23). In some studies, demonstration of *T.gondii* antigens in serum samples of experimentally infected animals suggests that antigens are detectable in acute phase of toxoplasmosis (24-7). For example, Raizman and Neva showed presence of circulating antigens in mice sera by counter-current electrophoresis and agar gel diffusion on day 2-4 of infection 28 (27). Shojaee *et al*. detected antigenemia from day 4 after infection in mice sera by immunoblotting (25).

Detection of *T.gondii* antigens in urine samples is a useful and non-invasive procedure for diagnosis of acute infection (15, 17-19). Huskinson *et al*. could demonstrate *T.gondii* antigens in sera and urine of mice as early as 5 days post infection by western blot (15).

Antigenemia in this study was apparent in 100% of experimentally infected cases, by capture ELISA. In addition, *T.gondii* antigens were detected from day two of infection in urine of mice. Regarding to high specificity and sensitivity of capture ELISA, this method could be used for diagnosis of acute toxoplasmosis in human especially for those in whom the infection may be widely disseminated. In this method rabbit immunization, preparation of polyclonal anti-rabbit antibody and conjugation has especial importance.

In conclusion, capture ELISA is a useful diagnostic method for detection of toxoplasmosis in mice. Because antigenemia occurs in a short period in acute phase of infection, clinical diagnosis of toxoplasmosis in acute phase is more difficult in human. Thus, altogether it is worthwhile to accomplish more serious and comprehensive studies for detection of antigenic components of *T.gondii* in human.

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