

## TECHNIQUE FOR THE DETECTION OF *TOXOPLASMA GONDII* ANTIGENS IN MOUSE URINE

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*A simple and rapid staphylococcal coagglutination test for the detection of Toxoplasma gondii antigens in mice urine is described. A suspension of protein-A containing Staphylococcus aureus coated with rabbit hyperimmune serum was used as reagent. The sensitivity of the antigen assay was found to be at least 118 ng of the antigen protein per ml. No coagglutination was observed when the reagent was challenged against antigenic solutions of other parasites. The suitability of the method for detecting antigens of T. gondii in urine samples was studied by experimental toxoplasma infection in mice. Before the staphylococcal test, the urine samples were double serially diluted in 0.1 M PBS. From the second day on all samples from infected mice were positive at 1/16 dilution. At this dilution, all samples from non infected mice were negative or did not produce coagglutination. This method might be used in the rapid etiological diagnosis also in human cases of acute toxoplasmosis.*

Key words: acute toxoplasmosis – *Toxoplasma gondii* – antigens – coagglutination test

The detection of causative agents of infectious diseases, offers the possibility of a safe and reliable diagnosis. Highly sensitive immunoassay techniques, such as enzyme-linked immunosorbent assay (ELISA), have been introduced recently, which are useful for the detection of antigenic components of various pathogens (Araújo & Remington, 1980; Linderschmidt, 1985; Knapen et al., 1986).

The diagnosis of acute toxoplasmosis is mainly based on the demonstration of the occurrence, or significative increased levels, of specific antibodies of either class A, class M and/or class G (Decoster et al., 1988).

However, these methods may occasionally lead to non conclusive results, such as patients with immunosuppressive diseases (Turunen, 1983). In such cases, the detection of *T. gondii* antigenic components in the body fluids can demonstrate the development of the disease (Raizman & Neva, 1975).

Various immunological methods have been used for the detection of *T. gondii* circulating

antigens, such as counter-immunoelectrophoresis, agar gel immunodiffusion and ELISA (Ise et al., 1985).

Coagglutination, in which antisera are adsorbed to protein A-containing Staphylococci, is a rapid, accurate and readily implemented diagnosis technique, which requires minimum resources for its use. It is also characterized by good sensitivity and specificity (Skaug et al., 1983).

The technique of coagglutination has a wide application spectrum. Initially, Christensen et al., (1973), Hahn et al., (1976) and Finch et al., (1977) used it for the detection and identification of *Streptococcus* serogroups, because it was safe, quick and simple. This method has also been successfully used to detect infections with *Salmonella* sp., *Neisseria meningitidis* or rotavirus. Recently, Kilvington & White (1986) used this method for the detection of *Entamoeba histolytica* and *Naegleria* antigens. In this latter case, the technique have been used to differentiate species.

This paper establishes the ability of this technique to detect antigens in mouse urine, infected with *T. gondii* trophozoites.

## MATERIALS AND METHODS

*Production of the soluble antigenic extract* — A soluble antigenic extract was prepared from the RH strain of *T. gondii*. The trophozoites were taken from peritoneal exudates of mice, three days after infection. The exudate was centrifuged at 650 g for 10 min at 4°C in 2 ml of 0.1M phosphate buffer saline (PBS), pH 7.2, to eliminate host cell residues. The supernatant was discarded and the pellet resuspended with 2 ml PBS and repeatedly passed through a 26 gauge needle in order to break the macrophages. It was again centrifuged at 160 g during 10 min at 4°C and the supernatant centrifuged at 650 g during 10 min at 4°C. The pellet was washed three times in 50 ml PBS. The suspension was then sonicated at 4°C to kill the parasite, and centrifuged at 650 g during 2 h at 4°C. The protein concentration of the supernatant was determined by the method of Lowry et al. (1951), and the *T. gondii* antigen stored at 20°C in 1 ml aliquots.

*Production of antiserum* — The anti-*Toxoplasma* antiserum was prepared by dilution of 0.5 ml of the *T. gondii* antigen (4 mg/ml of protein) in the same volume of PBS. The diluted antigenic solution was mixed in an equivalent volume of Freund's complete adjuvant for subcutaneous inoculation into rabbits (approximately 3 kg body weight).

Four inoculations were performed at weekly intervals. Seven days after the final inoculation, the animals were bled by cardiac puncture and the sera stored at -20°C. The anti-*Toxoplasma* antibody titre in the hyperimmune sera was determined by counterimmunoelectrophoresis (1/64), and ELISA (1/51200).

*Preparation of sensitized Staphylococcus aureus* — *S. aureus*, strain Cowan I, was grown in a modified "ccy" broth (Arvidson et al., 1971). The bacteria were fixed with 2% formaldehyde in PBS and heat-treated according to the method described by Jonsson & Kronvall (1974). Immediately before sensitization with antibodies, the bacteria were washed 3 times in PBS. 1 ml of a 10% (w/v) *S. aureus* suspension in PBS was mixed with 0.5 ml of the anti-*Toxoplasma* rabbit hyperimmune serum and was kept for three hours at 37°C. The antibody-coated *Staphylococci* were washed and resuspended to a final suspension of 5% (W/V) in

PBS containing 0.4% methylene blue and 0.1% sodium azide. *Staphylococci* coated with the rabbit preimmune serum were used as a control reagent.

*Urine sample* — Fifty female balb/c mice with approximately 20 g body weight were used to obtain the urine samples. Of these, 40 were inoculated with the *T. gondii* RH strain and 10 were used as negative controls. Mice were inoculated intraperitoneally with  $5 \times 10^6$  *T. gondii* trophozoites in 0.1 ml PBS.

*Sensitivity and Specificity of the test* — To assure adequate sensitivity, the staphylococci coated with anti-*Toxoplasma* rabbit hyperimmune serum were control tested against homologous antigen diluted in a double serial manner in urine (4 mg/ml; 2 mg/ml; . . . ; 59 ng/ml). To discard crossreactivity, this coagglutination reagent was control tested against heterologous antigens diluted in urine (*G. lamblia*, *E. histolytica*, *T. canis*, *Exc-Sec Ag F. hepatica*, *somatic Ag F. hepatica*, *A. cantonensis*, *D. immitis*).

Urine was collected during four consecutive days after inoculation of the mice. In order to confirm the effectiveness of the inoculation, the presence of *T. gondii* in the peritoneal cavity of mice died five days after inoculation, was verified.

To determine the working dilution, double serial dilutions were prepared with urine taken from both: infected and non infected mice.

*The coagglutination test* — For the coagglutination test, two separate drops of a antigenic solution or a urine sample were placed on white cardboard. A drop of the suspension of *staphylococci* coated with hyperimmune serum was added to one of the drops and a drop of the control reagent to the other. The white cardboard was rotated manually for 2 min, and coagglutination results were graded individually by three operators as 4x, 3x, 2x, 1x and negative, based on size of the clumps and clarity of the mixture.

## RESULTS

The coagglutination system described in this paper was able to detect *T. gondii* antigens at concentrations ranging from 4 mg/ml to 118 ng/ml. Lower concentrations did not produce visible agglutination of *staphylococcal*

TABLE I

Determination of the working dilution of urine for the detection of *Toxoplasma gondii* antigens by coagglutination test

Sensitized <i>Staphylococcus</i>		< 1/16		≥ 1/16	
		Positive	Negative	Positive	Negative
Urine from infected mice	Hyperimmune serum	40	Negative	40	Negative
	Preimmune serum	16	24	Negative	40
Urine from non infected mice	Hyperimmune serum	6	4	Negative	10
	Preimmune serum	6	4	Negative	10

particles. When the test was used against antigenic solution of other parasites no agglutination occurred.

Once the sensitivity and specificity of the coagglutination technique were known, it was necessary to determine the working dilution of the urine samples. As it can be seen in Table I, the optimum dilution was 1/16, because it avoid the presence of false positive results.

Table II shows the distribution of the intensity of coagglutination according to the days after inoculation with *T. gondii*. The presence of *T. gondii* antigen in urine can be detected by this technique from the first up to the fourth day of inoculation when mice died with acute toxoplasmosis.

TABLE II

Distribution of the coagglutination intensity scores according to the time of *Toxoplasma gondii* infection

Days after inoculation	Intensity CoA			
	1	2	3	4
Neg	35			
1x	5	22		
2x		18	16	
3x			24	12
4x				28

DISCUSSION

Raizman & Neva (1975), determined the presence of circulating *T. gondii* antigens in sera from both mice and rabbits. In mice, these antigens were detected two to four days after infection, by counterimmunoelectrophoresis

and agar gel diffusion techniques. These techniques were not suitable for testing rabbit sera but soluble antigens could also be identified in rabbits on days 3, 5, 7 and 8 of infection using cyanogen bromide-activated affinity chromatography.

Linderschmidt (1985) used an ELISA for detection of soluble antigens in sera from patients with acute toxoplasmosis. These antigens were found to circulate for a shorter period of time than the specific IgM antibodies. Thus, it was suggested that the detection of the antigens could be better indicator of a recent infection.

Turunen (1983), described an ELISA capable of detecting soluble *T. gondii* antigens in mouse urine from the second day of infection on.

The coagglutination technique developed by us for detecting soluble *T. gondii* in mouse urine was practically as sensitive as the Turunen system (118 ng/ml). It was able of detecting *T. gondii* antigens on the first day after inoculation in five of the forty infected animals. Its specificity was demonstrated by the absence of agglutination when the *staphylococci* coated with anti-*toxoplasma* hyperimmune serum were mixed with antigenic preparations from other parasites.

The coagglutination method described in this paper is inexpensive and the reading of the test does not require special training of health personnel and thus can be performed under field conditions. In view of this and its high sensitivity and specificity, demonstrated here, studies are being undertaken in our laboratory to adapt the same technique in a test for detecting *T. gondii* antigens in urine of humans infected with this parasite.

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