PREDICTIVE VALUE OF LATEX AGGLUTINATION TEST IN SEROLOGICAL SCREENING FOR *TOXOPLASMA GONDII*

Yaowalark Sukthana, Thaiyooth Chintana, Waraporn Supatanapong, Chutatip Siripan, Amorn Lekkla and Rachatawan Cheabchalrad

Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand

Abstract. The predictive value of commercial latex agglutination kit (Toxo-Screen DA, bioMerieux) was assessed for use as screening test for *Toxoplasma* IgG antibody. The sensivity and specificity were also compared with those of the reference standard Sabin-Feldman dye test. Five hundred serum samples were collected from 200 blood donors and 100 each from pregnant women, kidney recipients and HIV infected persons.

Eighty (16.0 %) out of 500 subjects were positive for *Toxoplasma* IgG antibody by Toxo-Screen DA (bioMerieux) compared with 57 (11.4%) by Sabin-Feldman dye test. The sensivity and specificity of Toxo-Screen DA (bioMerieux) were 100 % and 94.8 % respectively which were similar to previous reports from the area of high prevalence of *Toxoplasma* infection. In present study the positive predictive value of Toxo-Screen DA (bioMerieux) was only 71.3%.

The latex agglutination test should be considered as a screening test for *Toxoplasma* antibody, especially by small laboratories in remote area due to its availability, simplicity, sensitivity and specificity. However, because of its moderate positive predictive value, the test should be used with caution in screening immunocompromised patients and pregnant women living in areas with low prevalence of *Toxoplasma* infection. Since the number of false seropositive cases would be relatively higher than in a highly prevalent area, confirmation by the dye test would be needed.

INTRODUCTION

Human *Toxoplasma* infection occurs worldwide. Usually asymptomatic in the clinically normal host, the intracellular protozoan parasite can cause disease if it infects immunodeficient subjects.

Since the advent of HIV/AIDS pandemic, concurrent *Toxoplasma* infection has become an important health problem in Thailand. Patients with toxoplasmic encephalitis were first documented in 1992 and the number of cases has been increasing annually, particularly from the northern part of the country (Anonymous, 1997). Among HIV positive and *Toxoplasma gondii* antibody-positive subjects, 43.2% had symptoms and signs of acute toxoplasmosis involving the eyes and/or the central nervous system. (Sukthana *et al*, 2000). Due to severe sequelae in those patients, prompt diagnosis and proper management are necessary.

The diagnosis of toxoplasmosis in the immunocompromised host is often inconclusive during the acute clinical manifestations. The presence of *Toxoplasma* antibody in the serum is one of the criteria used in making the presumptive diagnosis of toxoplasmosis in immunosuppressive subjects (Ho-Yen, 1992). Moreover, *Toxoplasma* antibody titer is regarded as a prognostic factor for the occurrence of toxoplasmic encephalitis (Hellerbrand *et al*, 1996; Derouin *et al*, 1996). Sabin and Feldman(1948) first described the dye test for the detection of *Toxoplasma*-specific antibodies. It is a highly specific and sensitive test

Correspondence: Yaowalark Sukthana.
Tel: (662) 683-9752; Fax: (662) 248-3189
E-mail: tmymv@mahidol.ac.th

and is still used as the reference method for the serodiagnosis of toxoplasmosis (Reiter-Owona *et al*, 1999). Since the method requires life antigen, only a reference laboratory can offer this test. In Thailand, to our knowledge, the dye test is available only at our laboratory. Alternative methods which are highly sensitive, easy to perform and not time-consuming are therefore needed. Latex agglutination, indirect hemagglutination and indirect immunofluorescence have been employed (Luppin and Powell, 1991; Guruz *et al*, 1996; Woldenmichael *et al*, 1998). Though latex agglutination test was found to be highly sensitive in previous studies from areas with high prevalence of *Toxoplasma* infection, it has never been assessed among population with comparatively low prevalence such as Thailand (Morakote *et al*, 1984; Malewong *et al*, 1989; Ho-Yen, 1992; Taechowisan *et al*, 1997; Chintatana *et al*, 1998; Sukthana 1999). The same highly sensitive method when applied in highly prevalent and low prevalent groups of population may yield different positive predictive value, which could be of significance. We therefore undertook the present study to find out whether latex agglutination test could be recommended for wider use for serological screening of *T. gondii* in populations with low prevalence of infection.

MATERIALS AND METHODS

A prospective cross-sectional study was performed in the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University from January to June 2000 as follows:

1. Five hundred serum samples were collected from:
- Two hundred HIV-negative blood donors.
- One hundred from each pregnant women, kidney transplant recipients and HIV-infected patients.

There were 312 males and 188 females. Their ages ranged from 16 to 67 years. All pregnant women were in their first pregnancy. Seventy-three of the 100 kidney recipients were in their first year after transplantation, whilst the remainder had had transplantation for more than one year. None of the 100 HIV-infected patients had clinical symptoms of toxoplasmosis reactivation.

2. IgG *Toxoplasma* antibody assay was performed on each serum sample using both the Toxo-Screen DA (bioMerieux) and the Sabin-Feldman dye test. Two technicians were involved; one performing the Toxo-Screen DA (bioMerieux) and the other the dye test. Neither person was aware of the other's result.

Toxo-Screen DA (bioMerieux)

This commercial test kit is used for the detection of anti-*Toxoplasma* IgG antibody by direct agglutination with sensitized antigen. In brief, formalin-treated *Toxoplasma* antigen agglutinates as a uniform mat when specific IgG antibody is present in the diluted serum. Non-specific agglutination is suppressed by using a diluted buffer containing 2-mercapto-ethanol to detect only IgG. The results were read after the reaction was left standing for 5 hours at room temperature. The test was positive if there was agglutination in the mat covering half of the well base, while the control antigen and the negative result showed the sediment as a spot or a small ring. The cut-off point was a titer of 1:40.

The Sabin-Feldman dye test

The test is based on complement-mediated cytolysis of antibody-coated live *T. gondii* tachypzoites indicated by their inability to take up methylene blue. It is highly specific and sensitive and is regarded as the reference method for the serodiagnosis of toxoplasmosis. We regard the value of > 4 IU or a titer of at least 1:16 to be positive as recommended by Reiter-Owona *et al* (1999).

3. Data were recorded and analyzed according to Sackett *et al* (1985) with respect to sensitivity, specificity and predictive value of Toxo-Screening DA (bioMerieux) test kit using the Sabin-Feldman dye test as the reference standard.

RESULTS

The IgG antibody to *T. gondii* was positive in 80 (16.0 %) out of 500 serum samples tested by Toxo-Screen DA (bioMerieux) whilst 57 (11.4%) out of 500 were positive by the dye test. Table 1 shows the result of positive and negative *Toxoplasma* antibody by the two methods. Twenty-three samples were positive for *Toxoplasma* antibody by Toxo-Screen DA (bioMerieux) but not by Sabin-Feldman dye test. Only 420 samples were negative for *Toxoplasma* antibody by Toxo-Screen DA (bioMerieux), compared with 443 samples by Sabin-Feldman dye test. The sensivity, specificity and positive predictive value of Toxo-Screen DA (bioMerieux) were 100 %, 94.8% and 71.3% respectively as shown in Table 1.

DISCUSSION

The presence of *Toxoplasma* antibody in the serum is regarded as the important criterion for the diagnosis of toxoplasmosis. At present, several laboratories utilize different techniques such as indirect hemagglutination, ELISA, indirect immunofluorescence and latex agglutination (Luppin and Powell, 1991; Guruz *et al*, 1996; Woldernichael *et al*, 1998). Ideally the test should be specific, sensitive and easy to perform even in a small laboratory.

Toxo-Screen DA (bioMerieux) which was used in the present study was similar to the test employed by Desmonts and Remington in 1980. We found the Toxo-Screen DA (bioMerieux) to be 100% sensitive, 94.8%

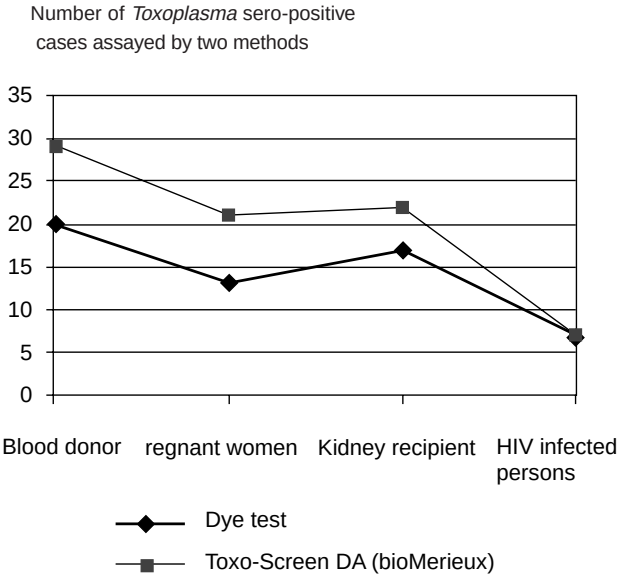

Fig 1–shows the seropositive of *T. gondii* using Toxo-Screen DA (bioMerieux) and Sabin-Feldman dye test in various groups of persons.

Table 1
shows the result of positive and negative *Toxoplasma* antibody assayed by the referent test (Sabin-Feldman dye test) and Toxo-Screen DA (bioMerieux).

Toxo-Screen DA(bioMerieux)	Sabin-Feldman dye test		Total
	Positive *Toxoplasma* antibody	Negative *Toxoplasma* antibody	
Positive *Toxoplasma* antibody	57	23	80
Negative *Toxoplasma* antibody	0	420	420
Total	57	443	500

Sensitivity of Toxo-Screen DA $= \frac{57 \times 100}{57} = 100\%$

Specificity of Toxo-Screen DA $= \frac{420 \times 100}{443} = 94.8\%$

Positive predictive value Toxo-Screen DA $= \frac{57 \times 100}{80} = 71.3\%$

specific, with a positive predictive value of 71.3%. The high sensitivity and specificity were similar to previous reports (Desmonts and Remington, 1980; Parker and Cubitt, 1992; Dubey *et al*, 1995; Guruz *et al*, 1996). Since the latex agglutination method is now commercially available and is very simple to carry out, it will be suitable for laboratories in remote areas when a serological test is only occasionally required. Moreover, Toxo-Screen DA (bioMerieux) can be used as a screening test to detect *Toxoplasma* antibody in immunocompromised patients such as those with HIV/AIDS and organ recipients with clinically suspected toxoplasma reactivation. Due to 100% sensitivity of the test, seronegative patients could be excluded, whilst seropositive cases need to be confirmed by the dye test since the positive predictive value of Toxo-Screen DA (bioMerieux) is only 71.3%. This is necessary because life-long chemoprophylaxis has to be given after treatment of acute toxoplasma reactivation.

In Thailand the sero-prevalence of *Toxoplasma* in pregnant women ranged from 2.8% to 21.7% (Morakote *et al*, 1984; Malewong *et al*, 1989; Taechowisan *et al*, 1997; Chintatana *et al*, 1998; Sukthana, 1999) and was lower than that in Western countries such as Austria (Sukthana, 1999). Our finding of positive predictive value of Toxo-Screen DA (bioMerieux) to be 71.3% indicates that the number of false positive cases would be significantly greater than that found in countries with high prevalence of toxoplasmosis. The test should thus be use with caution in countries with low prevalence of *Toxoplasma* infection in pregnant women such as Thailand, since seronegative pregnant women need counseling about risk factors that could cause primary *Toxoplasma* infection during pregnancy.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Saiyood Incheng and Miss Niramon Thima for their help in laboratory work. This study was financially supported by Thanat-Molee Khoman Foundation. The Toxo-Screen DA test kits were kindly provided by bioMerieux Company, France.

REFERENCES

Anonymous. Report of the opportunistic infection in HIV/AIDS patients in Chiang Rai Province. Chiang Rai Health Office, Ministry of Public Health, 1997.

Chintana T, Sukthana Y, Bunyakai, Lekkla A. *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. *Southeast Asian J Trop Med Public Health* 1998; 29: 383-6.

Derouin F, Leport C, Pueyo S, *et al*. Predictive value of *Toxoplasma gondii* antibody titres on the occurrence of toxoplasmic encephalitis in HIV-infected patients. ANRS 005/ACTG 154 Trial Group. *AIDS* 1996;10: 1521-7.

Desmonts G, Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: Method for increasing sensivity and specificity. *J Clin Microbiol* 1980; 11: 562-8.

Dubey JP, Thulliez P, Weigel RM, Anderws CD, Lind P, Powell EC. Sensitivity and specificity of various serological test for detection of *Toxoplasma gondii* in naturally infected sows. *Am J Vet Res* 1995; 56: 1030-6.

Guruz AY, Ok UZ, Korkmar M. Assessment of latex indirect agglutination test (Toxolatex Fumouze) for the detection of *Toxoplasma* specific antibody in human sera in Turkey. *J Egypt Soc Parasitol* 1996; 26: 376-84.

Hellerbrand C, Goebel FD, Disko R. High predictive value of *Toxoplasma gondii* IgG antibody levels in HIV-infected patients for diagnosis of cerebral toxoplasmosis. *Eur J Clin Microbiol Infect Dis* 1996; 15: 869-72.

Ho-Yen DO. Diagnosis of Toxoplasmosis. In: Ho-Yen DO, Joss AWL, eds. Human Toxoplasmosis. London: Oxford University Press, 1992.

Luppin MR, Powell CC. Comparison of latex agglutination, indirect hemagglutination, and ELISA techniques for the detection of *Toxoplasma gondii*-specific antibodies in the serum of cats. *J Vet Intern Med* 1991; 5: 299-301.

Malewong W, Lulitanand V, Pipitgool V, Auwijtaroon Y, Kuttsarejariya S, Morakote N. Prevalence of

Toxoplasma gondii antibodies in blood donor and pregnant women in Khon Kaen Province. *J Med Assoc Thai* 1989; 72: 256-9.

Morakote N, Thamasonthi W, Charuchinda K. Prevalence of *Toxoplasma* antibodies in Chiang Mai population. *Southeast Asian J Trop Med Public Health* 1984; 15: 80-5.

Parker SP, Cubitt WD. Modified latex agglutination test for antibodies to *Toxoplasma gondii* in elutes from Guthrie cards. *J Clin Pathol* 1992; 45: 907-9.

Reiter-Owona I , Petersen E, Joynson D, *et al*. The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. *Bull WHO* 1999; 77: 929-35.

Sabin AB, Feldman A. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*). *Science* 1948; 108: 660-3.

Sackett DI, Haynes RB, Tugwell P. The Interpretation of Diagnostic Data. Clinical Epidemiology a Basic Science for Clinical Medicine. Boston: Little, Brown and Company. 1985; 59-138.

Sukthana Y, Chintana T, Lekkla A. *Toxoplasma gondii* antibody in HIV-infected Persons. *J Med Assoc Thai* 2000; 83: 681-4.

Sukthana Y. Difference of *Toxoplasma gondii* antibodies between Thai and Austrian pregnant women. *Southeast Asian J Trop Med Public Health* 1999; 31: 38-41.

Taechowison T, Sutthent R, Louisirirotchanabul S, Puthawathana P, Wasi C. Immune status in congenital infection by TORCH agents in pregnant Thais. *Asian Pac J Allergy Immunol* 1997; 15: 93-7.

Woldemicheal T, Fontanet AL, Sahlu T, *et al*. Evaluation of the Eiken latex agglutination test for anti-*Toxoplasma* antibodies and seroprevalence of *Toxoplasma* infection among factory workers in Addis Ababa, Ethiopia. *Trans R Soc Trop Med Hyg* 1998; 92: 401-3.