# IMMUNOBLOTTING AND ENZYME LINKED-IMMUNOSORBENT ASSAY FOR DIAGNOSIS OF *TOXOPLASMA* INFECTION IN HIV THAI PATIENTS

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**Abstract.** *Toxoplasma gondii* has been recognized as an important cause of morbidity and mortality among immunocompromised persons. The diagnosis of *T. gondi* infection is most often based on serological tests results. Serological diagnosis can be limited in AIDS patients because of depressed antibody responses. Fifty serum samples were used in this study to investigate serological evidence of toxoplasmosis in HIV positive Thai patients by Platelia kit, the commercial enzyme-linked immunosorbent assay (ELISA) in which the membrane protein p-30 is the predominant antigen and immunoblot technique (IB). Sera of HIV positive Thai patients with *Toxoplasma* infection recognized the same antigenic component, the 32 kDa antigenic band, as is recognized by *Toxoplasma* positive sera from immunocompetent patients and it may represent a specific marker for diagnosis of *Toxoplasma* infection in HIV positive Thai patients.

## INTRODUCTION

*Toxoplasma gondii*, the causative agent of toxoplasmosis, has been recognized as an important cause of morbidity and mortality among immunocompromised persons. It is one of the most frequently encountered opportunistic infections in patients with the acquired immune deficiency syndrome (AIDS) (Holliman, 1988). The diagnosis of *T. gondii* infection is most often based on serological tests results such as indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA). False positive results have been reported in certain of these tests and serological diagnosis can be limited in AIDS patients owing to depressed antibody responses (Salata, 1990). Immunoblot (IB) is a technique that combines electrophoresis of toxoplasmic antigens under denaturing conditions, and electrotransfer, and is a specific antibody test (Towbin *et al*, 1979). IB has successfully been used to characterize the antigens to which antibodies are formed in adults with acute or latent toxoplasmosis (Partanen *et al*, 1984). The objective of the present study was to study the serological evidence of toxoplasmosis in the HIV positive patients from our previous study using Platelia, the commercial enzyme-linked immunosorbent assay kit in which the membrane protein p-30 is the predominant antigen. We also aimed to assess whether *Toxoplasma* infection in HIV infected patients could be diagnosed by appearance of characteristic antibodies reacted against specific *Toxoplasma* antigen by immunoblot analysis.

## MATERIALS AND METHODS

### Sera

50 serum samples were used in this study. The sera were obtained from 3 population groups as follows:

Group 1 consisted of 17 sera obtained from HIV infected patients with a positive *Toxoplasma* serology and 23 sera from HIV infected patients with negative *Toxoplasma* serology. (These sera were used in our previous study and have been kept at -70 °C in our laboratory).

Group 2 composed of 5 sera from patients with other parasitic infections or with other diseases such as rheumatoid arthritis, systemic lupus erythematosis.

Group 3 consisted of 5 sera from healthy blood donors, collected by random sampling method.

### Control sera

An international standard reference serum (provided per WHO by Statens Serum Institute, Copenhagen, Denmark) and serum from healthy person with negative *Toxoplasma* antibodies were used as positive and negative control serum respectively.

### Antigen for IB

Antigen for IB were prepared from the tachyzoites of *T. gondii* RH strain using the method of Sharma (Sharma *et al*, 1983). Its protein content was 43 mg/dl.

**Detection of IgG antibody to *T. gondii***

**Enzyme-linked immunosorbent assay (ELISA)**

All sera were examined for *Toxoplasma*-specific IgG antibodies by Platelia-Toxo-IgG purchased from Sanofi Diagnostic Pasteur, France according to the instruction of the manufacturer.

**Immunoblot technique**

The method of Towbin (Towbin *et al,* 1979) was modified as follows. Briefly, *T. gondii* somatic antigen was denatured for 2 minutes at 100°C with β-mercaptoethanol plus sodium dodecyl sulfate and separated by electrophoresis in a 13% polyacrylamide gel at 150 V, 0.12 A for 45 minutes at 25°C on the Bio-Rad-minigel. After electrophoresis, the gel was further blotted on 0.45 μm-pore-size nitrocellulose sheet (Costa) for 45 minutes at 6°C using the Bio-Rad Transblot system. Then the blotted membrane was blocked with 5% skimmed milk-PBS pH 7.2 for 30 minutes to avoid any nonspecific reaction, was cut in strips and was stored at -20°C until used. Diluted samples (dilution 1:50) were applied to the nitrocellulose strips and the strips were incubated at 25°C for 2 hours. The unbounded materials were removed by washing with phosphate buffer saline, pH 7.2 for 5 times. Anti-human IgG conjugated with horseradish peroxidase (dilution 1:1,000) was added to the strips, the strips were incubated at 25°C for 1 hour and were washed again as in the previous step. Bands were visualized by addition of 2-6 dichlorophenol and 2% $H_2O_2$ for 5 minutes. The reaction was stopped by washing the strips with distilled water. The molecular weights of the bands were calculated by comparison with standard markers.

## RESULTS

Antibodies to *T. gondii* were detected in 50 sera from the studied groups using ELISA and IB. For ELISA, 17 sera in group 1 gave a positive result and 23 sera from this group gave a negative result. Fig 1 shows the antigenic patterns of immunoblot analysis. *Toxoplasma* antibodies reacted with several polypeptides of the soluble *T. gondii* antigen over a wide molecular weight ranged from 21 to 105 kDa. Three major antigenic bands of 22, 28.5 and 32 kDa were recognized by the seropositive sera in group 1. The 32 kDa band was detected in 15 of 17 sera. The 22 and 28.5 kDa bands were detected in only 3 of 17 and 5 of 17 sera respectively. The seronegative sera from groups 2 and 3 did not reveal these bands.

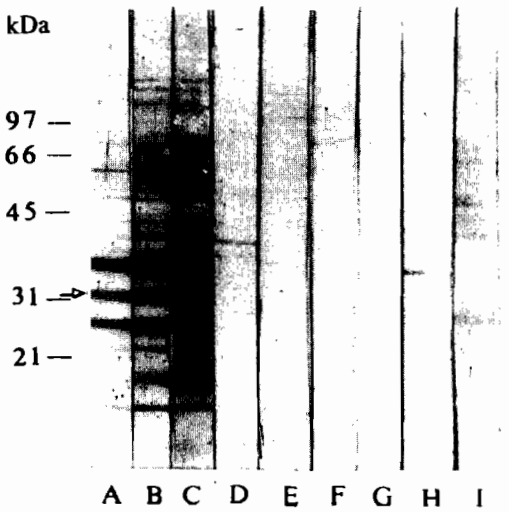


Fig 1–Immunoblot analysis of *T.gondii* antigens recognized by sera in the studied groups. Lane A: a toxoplasma positive control serum from WHO; lane B-C: sera from HIV infected patients with toxoplasmosis; lane D-E: sera from HIV infected patients without toxoplasmosis; F-G: sera from other diseases and lane H-I: sera from healthy persons. Arrow represented the 32 kDa band. Numbers represent molecular weight standard ($10^3$).

## DISCUSSION

An accurate diagnostic method for the detection of toxoplasmosis in HIV-infected patients is important in order to minimize morbidity and mortality by institution of prompt treatment. The results from our present study in IB demonstrated the presence of antigenic components which reacted with IgG antibodies to *Toxoplasma* in sera of HIV positive Thai patients. The intensely stained band with a molecular weight of 32 kDa was found in a majority of *Toxoplasma* positive sera. The polypeptides of 25, 32 and 35 kDa have been reported by many investigators to be the major *Toxoplasma* antigens recognized by IgG and IgM (Sharma *et al,* 1983; Potasma *et al,* 1986; Moir *et al,* 1991). The 32 kDa component may correspond to the major immunogenic surface antigen p-30 (Rodriguez *et al,* 1985; Santoro *et al,* 1985; 1986), since p-30 has been reported as lying between 27-35 kDa depending on the electrophoretic conditions (Kasper *et al,* 1983; Couvreur *et al,* 1988) and it can be used as a single antigen in serodiagnosis of both acute and chronic toxoplasmosis (Santoro *et al,* 1985). Louis and Stephen studied the antibody response of HIV positive patients by Western blot and the major antigen

has been recognized by his studies was 35 kDa which was said to be the major surface protein of *T. gondii*, p-30 (Louis *et al*, 1988). Thus our result agreed with other investigators. Toxoplasmosis in the immunocompromised host is considered to be the result of reactivation of chronic infection (Luft *et al*, 1984). Decoster stated that the 28.5 kDa antigen seemed to be characteristic of the chronic phase of toxoplasmosis (Decoster *et al*, 1988). But from our study, the 28.5 kDa was found only in 29.4% of *Toxoplasma* seropositive sera.

Although the detection of IgG antibody to *T. gondii* has been discussed by some investigators as having limited value in the diagnosis of *Toxoplasma* reactivation in HIV positive patients, it may be important in predicting prognosis (Rodriguez *et al*, 1997). But the detection of *Toxoplasma*-specific IgM or IgA which could indicate recent infection, are unreliable because these antibodies were not persistence (Wong *et al*, 1994). Recently, detection of *T.gondii* circulating antigen has been studied for the diagnostic purposed in HIV positive patients and *Toxoplasma* encephalitis. But its usefulness as a diagnostic tool is limited due to its low sensitivity and specificity (Wong *et al*, 1994). For ELISA, all *Toxoplasma* positive and negative sera in group 1 gave concordance with the result from our previous study (Wongkamchai *et al*, 1995).

In conclusion, sera of HIV positive Thai patients with *Toxoplasma* infection recognized the same antigenic component as recognized by *Toxoplasma* positive sera from immunocompetent patients. The present results could also confirm our previous finding about the positive rate of toxoplasmosis in the HIV infected patients which seems quite high (Wongkamchai *et al*, 1995).

## REFERENCES


Couvreur G, Sadak A, Fortier B, Dubremetz JF. Surface antigens of *T. gondii*. *Parasitology* 1988; 97: 1-10.

Decoster A, Darcy F, Capron A. Recognition of *Toxoplasma gondii* excreted and secreted antigens by human sera from acquired and congenital toxoplasmosis: identification of markers of acute and chronic infection. *Clin Exp Immunol* 1988; 73: 376-82.

Holliman RE. Toxoplasmosis and the acquired immune deficiency syndrome *J Infect* 1988; 16: 121-8.

Kasper LH, Crabb JH, Pfefferkorn ER. Purification of a major membrane protein of *T. gondii* by immunoabsorption with a monoclonal antibody. *J Immunol* 1983; 130: 2407-12.

Louis MW, Stephen AU. Western blot analysis of the antibody response of patients with AIDS and *Toxoplasma* encephalitis: Antigenic diversity among *Toxoplasma* strain. *J Infect Dis* 1988; 157: 7-13.

Luft BJ, Brooks RG, Conley FK, McCabe RE, Reminton JS. Toxoplasmic encephalitis in patients with acquired immune deficiency syndrome. *JAMA* 1984; 252: 913-7.

Moir IC, Davidson MM, Hoyen DO. Comparison of IgG antibody profiles by immunoblotting in patients with acute previous *T. gondii* infection. *J Clin Pathol* 1991; 44: 569-72.

Partanen P, Turunen HJ, Paasivuo RTA, Leinikki PO. Immunoblot analysis of *T. gondii* antigens by human immunoglobulins G, M and A antibodies at different stages of infection. *J Clin Microbiol* 1984; 20: 133-5.

Potasma I, Araujo FG, Desmonts G, Remington JS. Analysis of *T. gondii* antigens reconized by human sera obtained before and after acute infection. *J Infect Dis* 1986; 154: 650-7.

Rodriguez C, Afchain D, Capron A, Dissous C, Santoro F. Major surface protein of *T. gondii* contains an immunodominant region with repetitive epitopes. *Eur Immunol* 1985; 15: 747-9.

Rodriguez JC, Martinez MM, Martinez AR, Royo G. Evaluation of different techniques in the diagnosis of Toxoplasma encephalitis. *J Med Microbiol* 1997; 46: 597-601.

Salata RA. Toxoplasmosis. In: Warren KS, Mahmoud AAF, eds. Tropical and geographical medicine. New York: McGraw-Hill Press, 1990: 36-41.

Sharma SD, Mullenax J, Araujo FJ, Erlich HA and Remington JS. Western blot analysis of the antigens of *T. gondii* recognized by human IgM and IgG antibodies. *J Immunol* 1983; 131: 977-83.

Santoro F, Afchain D, Pierce R, Cesbron JY, Ovlaque G, Capron A. Serodiagnosis of *Toxoplasma* infection using purified parasite protein (p-30). *Clin Exp Immunol* 1985; 62: 262-9.

Santoro F, Charif H, Capron A. The immunodominant epitope of the major membrane tachyzoite protein (p-30) of *T. gondii*. *Parasite immunol* 1986; 8: 631-5.

Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and source applications. *Proc Natl Acad Sci USA* 1979; 76: 43-50.

Wong SY, Reminton JS. Toxoplasmosis in pregnency. *Clin Infect Dis*. 1994; 18: 853-62.

Wongkamchai S, Rungpitaransi B, Wongbunnate S, Sittapairochana C. *Toxoplasma* infection in healthy persons and in patients with HIV or ocular disease. *Southeast Asian J Trop Med Public Health* 1995; 26: 655-7.