

IDENTIFICATION OF *Toxoplasma gondii* ANTIGENS INVOLVED IN THE IgM AND IgG INDIRECT HEMAGGLUTINATION TESTS FOR THE DIAGNOSIS OF TOXOPLASMOSIS

Y.I. YAMAMOTO (1), V. HUBER (1) & S. HOSHINO-SHIMIZU (1, 2)

SUMMARY

Crude *Toxoplasma gondii* antigens represent raw material used to prepare reagents to be employed in different serologic tests for the diagnosis of toxoplasmosis, including the IgM and IgG indirect hemagglutination (IgG-HA and IgM-HA) tests. So far, the actual antigenic molecules of the parasite involved in the interaction with agglutinating anti-*T. gondii* antibodies in these tests are unknown. The absorption process of serum samples from toxoplasmosis patients with the IgG-HA reagent (G-toxo-HA) demonstrated that red cells from this reagent were coated with *T. gondii* antigens with Mr of 39, 35, 30, 27, 22 and 14 kDa. The immune-absorption process with the IgM-HA reagent (M-toxo-HA), in turn, provided antibody eluates which recognized antigenic bands of the parasite corresponding to Mr of 54, 35 and 30 kDa, implying that these antigens are coating red cells from this reagent.

The identification of most relevant antigens for each type of HA reagent seems to be useful for the inspection of the raw antigenic material, as well as of reagent batches routinely produced. Moreover the present findings can be used to modify these reagents in order to improve the performance of HA tests for the diagnosis of toxoplasmosis.

KEYWORDS: *Toxoplasma gondii* antigen; Hemagglutination test; Antibody absorption; Antibody elution; Immunoblot assay.

INTRODUCTION

Toxoplasmosis is a ubiquitous infection caused by *Toxoplasma gondii*, an intracellular protozoan parasite of worldwide distribution. The infection is usually mild or asymptomatic in immunocompetent subjects but severe disease may occur in infected fetuses and immunocompromised individuals^{14,24}.

The serodiagnosis of toxoplasmosis relies mostly on the detection of IgG, IgM and, more recently IgA antibodies, with tests such as indirect hemagglutination (HA), immunofluorescence (IF) and enzyme linked immunosorbent assay (ELISA)^{2,4,5,14,24}.

The features of crude *Toxoplasma gondii* antigens have been extensively investigated^{7,9,18,23}, but the antigens actually involved in serological tests carried out to capture antibodies, which are the immunological markers for the diagnosis of toxoplasmosis, have not been investigated in depth.

The conventional reagent for the IgG-HA toxoplasmosis test is routinely prepared^{2,5} in public health laboratories which produce several homemade immunodiagnostic reagents, although this type of reagent is also commercially available. It is obtained by treating red blood cells with tannic acid and sensitizing them with crude *T. gondii* antigen. A hemagglutination test for the detection of IgG antibodies (G-toxo-HA) and another for IgM detection (M-toxo-HA) utilizing different types of antigens were recently described^{24,25}. The reagent G-toxo-HA is prepared with an antigen which is first submitted to alkaline solubilization and neutralization, followed by the usual sensitization process with tannic acid. This reagent was shown to be as sensitive as the above conventional reagent for the IgG-HA test. In contrast, the M-toxo-HA reagent uses the same antigen but previously heated at 121°C to denature the proteins. The red cells are then sensitized by direct contact with this antigen. In both processes for reagent preparation, red cells

(1) Departamento de Análises Clínicas e Toxicológicas da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, São Paulo, SP, Brasil.

(2) Setor de Imunologia do Instituto Adolfo Lutz, São Paulo, SP, Brasil.

Correspondence to: Yoshimi Imoto Yamamoto, Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas da USP, Av. Prof. Lineu Prestes 580 bloco 17, 05508-900 São Paulo, SP, Brasil

seem to select several parasite components from the crude antigen, and therefore these reagents are thought to present an antigen spectrum differing from that of the raw material.

Thus, in the present study we identified the main antigens responsible for agglutination in the G-toxo-HA and M-toxo-HA tests by an immunoblot assay after submitting sera from toxoplasmosis patients to the processes based on antibody absorption or antibody elution.

MATERIAL AND METHODS

Toxoplasma antigen

Toxoplasma gondii tachyzoites, RH strain, were obtained from mice as described previously^{14,25}. Briefly, 2×10^9 parasites were solubilized in 2.5 ml 0.15 N NaOH at 4°C for 6 h. After neutralization with 0.3 N HCl solution, the antigen was divided into two parts. One part corresponded to the Alk-G antigen and the other part, which was heated at 121°C for 20 min in an autoclave, was denoted Alk-M antigen. Both antigens were centrifuged at 320 g for 10 min to remove insoluble residues and kept at -20°C until use.

G-toxo-HA and M-toxo-HA reagents

The G-toxo-HA reagent was prepared as reported by CAMARGO et al.³ using tanned red cells and Alk-G antigen. The M-toxo-HA reagent was prepared as described^{24,25} by directly sensitizing red cells with Alk-M antigen.

Serum samples

Serum samples were collected from three patients with acute toxoplasmosis presenting serologic profile I² for IgM and IgG specific antibodies, and from three individuals with chronic toxoplasmosis with serologic profile III for IgG antibodies. In addition, serum samples from two individuals serologically negative for toxoplasmosis were included in the study as negative controls.

Immunologic techniques

Serum samples were tested by IgG immunofluorescence (IgG-IF)² and by indirect hemagglutination (IgG-HA)⁵ before and after absorption with G-toxo-HA reagent. They were also tested by IgM-IF and IgM-HA^{2,25} before absorption with the M-toxo-HA reagent, as well as with the antibodies eluted from this reagent. All serum samples before and after absorption as well as antibodies eluted from the reagents were submitted to the immunoblot assay (IBA).

Antibody absorptions

Six ml of G-toxo-HA reagent were washed in saline and 1 ml of serum at 1:100 dilution in saline (NaCl 0.15 M) containing 0.5% skim milk was added to the sediment. After incubating for one hour at room temperature with slow shaking, the mixture was left to stand for an additional hour, and then centrifuged at 1,000 g, and the supernatant was assessed by immunologic techniques.

Antibody elutions

Forty ml of M-toxo-HA reagent were washed in saline and 80 ml serum at 1:80 dilution in 0.10 M NaCl containing 0.5% skim milk were added to the sediment. The mixture was

incubated at 37°C for 90 min and then at room temperature for 2 h. Red cells were washed twice with saline containing 0.5% skim milk, and antibodies were eluted with 1 ml of 0.1 M glycine-NaOH, pH 10.5, at 37°C for 4 min¹¹. Immediately after elution, the mixture was centrifuged and the supernatant was neutralized with 0.5 M Tris, pH 7.2, not exceeding a volume of 2 ml. The antibody eluates were then assessed by immunologic techniques. Antibodies were also eluted under acid conditions as described above, except that with 0.1 M glycine-HCl, pH 2.5, and the eluates neutralized with 0.15 N NaOH.

Immunoblot assay (IBA)

The *T. gondii* antigen was denatured as described⁶. Briefly, protein components were separated by electrophoresis on 12% gel¹, including low molecular mass markers (Phosphorilase B - 94kDa, bovine albumin - 67kDa, ovalbumin - 43 kDa, carbonic anhydrase - 30 kDa, trypsin inhibitor - 20.1 kDa and α lactalbumin - 14.4 kDa). The proteins were transblotted (Multiphor, Pharmacia LKB Biotechnology, NJ, USA) to 0.45 μ -pore-size nitrocellulose sheets (Millipore, Bedford, MA, USA) for 90 min at 0.8 mA/cm² and saturated in 0.1 M PBS-5% skim milk, pH 7.2, for 2 h at room temperature. Absorbed and nonabsorbed serum samples from patients with chronic and acute toxoplasmosis, and negative serum were diluted 1:50 in 0.01 M PBS-1% skim milk, pH 7.2, (PBSM) and added to the nitrocellulose sheets for 2 h at room temperature with mechanical shaking. The antibody eluates were tested without dilution. After four washes of 10 min in PBS, goat anti-human IgG (1:1000) and IgM (1:400) peroxidase conjugates (SIGMA, St. Louis, MO, USA) diluted in PBSM were added, and the membranes incubated for 30 min at 37°C. After a new cycle of washings, bands were visualized with 0.017% diaminobenzidine plus 0.15% H₂O₂ in PBS. The reaction was stopped by two washes in distilled water. The Mr of the bands were determined using the DNA Star Program (Computed Systems for Molecular Biology and Genetics, CDC).

RESULTS AND DISCUSSION

The results reported here about the identification of *T. gondii* antigenic components of the G-toxo-HA and M-toxo-HA reagents provide the basis for further improvements in the performance of the hemagglutination tests for the diagnosis of toxoplasmosis. Also, in the quality control analysis, it is essential to inspect the main antigenic components in raw antigenic materials before the preparation of the reagent, and thereafter in successively produced reagents.

Although the data here obtained are still preliminary, the two processes of antibody absorptions and antibody elution permitted us to identify the antigenic components of *T. gondii* actually involved in the capture of agglutinating antibodies.

The results obtained in sera from patients with chronic toxoplasmosis before and after antibody absorption using the G-toxo-HA reagent are illustrated in table 1. This process was shown to be efficient since the absorbed sera, when retested along with the nonabsorbed sera, presented remarkable

TABLE 1

Antibody levels detected by serologic tests in serum samples from chronic toxoplasmosis patients before and after absorption with the G-toxo-HA reagent.

Serologic Tests	Serum Sample N°	Antibody Titers	
		Before Absorption	After Absorption
G-toxo-HA	1	2560	< 40
	2	1280	< 40
	3	2560	20
	4	5120	160
	NC	NR	NR
IgG-IFI	1	256	< 40
	2	256	< 40
	3	256	64
	4	1024	256
	NC	NR	NR

NR: not reactive

NC: negative control serum

differences in the serologic tests, giving negative results or showing a significant decrease in antibody titer.

Data from the process of antibody elution are presented in table 2. Serum samples from patients with acute toxoplasmosis were absorbed with the M-toxo-HA reagent, and the IgM antibodies eluted from this reagent under alkaline conditions gave better results in comparison to the conventionally acid elution⁸. This finding agrees with that reported by LIM¹¹ who worked with the purification of monoclonal IgM antibodies. The IgM antibody titers of the sera (Table 2) were high in contrast to the low titers of the IgM antibody eluates, showing a considerable loss of antibodies during the elution process. The loss of antibodies is expected because several factors interfere not only with the elution and washing steps but also because of antibody denaturation. The estimated yield of IgM antibodies in the eluates was about 20%, and reproducible for all sera submitted to the elution process. LIM¹¹ also obtained a similar yield (38%) in the purification of monoclonal IgM, as evaluated by an immunoenzymatic assay.

The efficiency of the antibody absorption and of the elution process was checked by the HA and IF tests. The latter test confirmed the HA test, implying that most *T. gondii* antigenic components are common to both tests. In the IF test, the results are interpreted based on the reactivity of antibodies with the parasite membrane. So it is possible to infer that, in the HA test, most antigenic components are predominantly from the parasite membrane.

A total of 18 antigenic bands of *T. gondii* were recognized by IgG antibodies from patients with chronic toxoplasmosis, corresponding to Mr of 93, 82, 76, 63, 58, 54, 50, 45, 43, 39, 35, 30, 27, 24, 22, 20, 16 and 14 kDa. In general, strong reactivities for 35 and 27 kDa were observed with IgG antibodies (Table 3). After absorption with G-toxo-HA reagent, serum samples

recognized three bands of 63, 58, and 54 kDa, with an intensity close to that of bands recognized by nonabsorbed sera. Thus, these antigenic bands are not selected by tanned red cells during the process to prepare the G-toxo-HA reagent.

The same absorbed serum samples, however, recognized bands of 43, 35, 30, 27 and 24 kDa in different fashions since some sera stained these bands weakly and others stained them in the same manner as the nonabsorbed sera. This means that some sera have low levels of IgG antibody to those bands and others have high

TABLE 2

Antibody levels detected by serologic tests in serum samples from acute toxoplasmosis patients and eluates from the M-toxo-HA reagent.

Serologic Tests	Serum Samples	Antibody Titers	
		Antibodies from serum	Antibodies eluted from the reagent
M-toxo-HA	6	1280	16
	7	2560	16
	8	1280	8
	NC	NR	NR
IgM-IFI	6	1024	16
	7	1024	nd
	8	1024	nd
	NC	NR	NR

NR: not reactive

NC: negative control serum

nd: not done

TABLE 3

Antigenic bands of *Toxoplasma gondii* recognized by IgG antibodies from chronic toxoplasmosis patients before and after absorption with the G-toxo-HA reagent, in the immunoblot assay.

Serum Sample No.	IgG Antibodies	
	Reactive Bands Before Serum Absorption (kDa)	Reactive Bands After Serum Absorption (kDa)
1	63, 58, 54, 50, 43, 39, 35*, 30, 27*, 24, 22, 20, 14	63, 58, 54, 50 ^f , 43 ^f , 35, 27, 24 ^f
2	63, 58, 54, 45, 43, 39, 35*, 30, 27, 14	63, 58, 54, 43 ^f , 35, 30 ^f
3	63, 58, 54, 50, 45, 43, 39, 35*, 30, 27, 24, 22, 14	63, 58, 54, 27 ^f
4	93, 82, 76, 54, 50, 43, 39, 35*, 30, 27*, 24*, 22, 20, 16, 14	54 ^f , 50 ^f , 43 ^f , 35, 27, 24 ^f
NC	no bands	no bands

NC: negative control serum

*: strongly stained bands

f: faintly stained bands

levels. Thus, one absorption was not sufficient to determine a detectable decrease in antibodies in sera with high antibody levels.

The IgG antibodies were also eluted from the G-toxo-HA reagent with acid solutions and bands of 35 and 27 kDa were observed. Considering the results as a whole, we conclude that antibodies to bands of 93, 82, 76, 45, 39, 35, 30, 27, 24, 22, 16 and 14 kDa were absorbed with the G-toxo-HA reagent. Thus, the G-toxo-HA reagent is composed of antigen epitopes found in the above 12 bands. However the epitopes found in six antigenic bands (39, 35, 30, 27, 22 and 14 kDa) are predominantly recognized by most sera from chronic toxoplasmosis patients.

IgM antibodies from patients with acute toxoplasmosis recognized a total of 13 bands of 91, 84, 74, 69, 64, 60, 54, 50, 47, 35, 32, 30 and 18 kDa, with the bands of 54, 35, 32, 30 and 18 kDa being strongly reactive. The IgM eluates from the M-toxo-HA reagent recognized seven bands of 69, 60, 54, 35, 32, 30 and 18 kDa corresponding to about 50% of the total bands recognized by the original sera (Table 4). The IgM eluates recognized consistently the band of 30 kDa.

Figure 1 illustrates immunoblot profiles of some of serum samples employed in the present study.

In the present work, *T. gondii* bands corresponding to antigens coating red cells were qualitatively identified by IB. However, it is possible to quantify the intensity of stained antigenic bands in a densitometric process. Figure 2 is an example of the densitograms (Fluor Densitometer, Model CS-9301PC, Shimadzu, Japan) obtained from the immunoblot strips with bands recognized by a serum (sample no.1) from chronic toxoplasmosis patient, before (I) and after (II) absorption with G-toxo-HA reagent. Most of areas (mm²) corresponding to peaks a, b, c, d, e, f, g, h, i, j, k + l and m from the pattern I decreased after absorption (pattern II), and these decreases

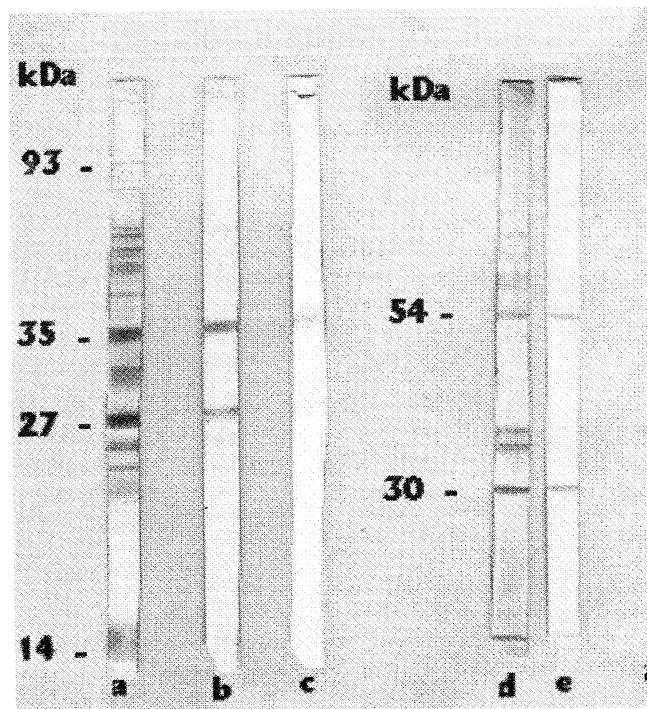


Fig 1 - Antigenic bands of *T. gondii* recognized by IgG antibodies from a chronic toxoplasmosis patient (serum no. 4): before absorption (a), after absorption with G-toxo-HA reagent (b) and IgG antibodies eluted from G-toxo-HA reagent (c). Antigenic bands of *T. gondii* recognized by IgM antibodies from an acute toxoplasmosis (serum no. 7) before absorption with M-toxo-HA reagent (d), and IgM antibodies from M-toxo-HA reagent (e)

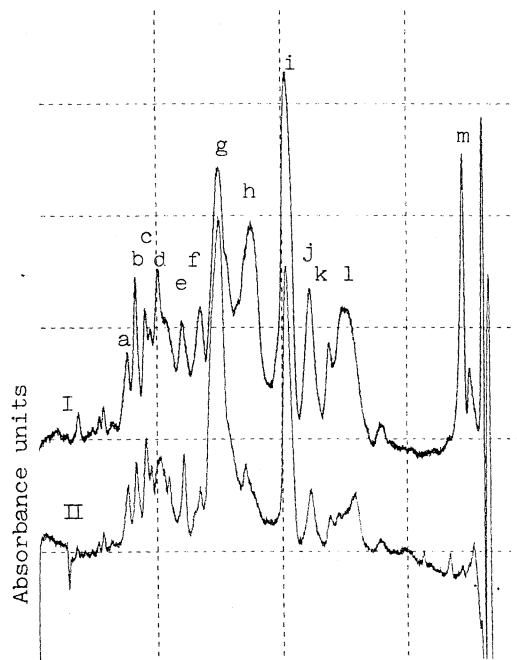


Fig 2 - Densitometric analysis of IgG pattern from a chronic toxoplasmosis patient (serum no. 1) before absorption (I) and after absorption with G-toxo-HA reagent (II) by immunoblot assay. Antigenic bands (kDa): a = 63; b = 58; c = 54; d = 50; e = 43; f = 39; g = 35; h = 30; i = 27; j = 24; k = 22; l = 20 and m = 14

TABLE 4

Antigenic bands of *Toxoplasma gondii* recognized in the immunoblot assay by IgM antibodies from acute toxoplasmosis patients and IgM antibodies eluted from M-toxo-HA reagent.

Serum Sample No.	IgM Antibodies	
	Reactive Bands with Sera (kDa)	Reactive Bands with Eluates from Reagent (kDa)
6	91, 84, 69, 60*, 54, 50, 47, 35*, 32*, 30*, 18*	69, 60*, 35, 32, 30*
7	74, 69, 64, 54*, 35, 32*, 30*, 18	54*, 30*
8	54, 35*, 32, 30*, 18	54, 35, 32, 30*, 18
NC	no bands	no bands

NC: negative control serum

* strongly stained bands

were estimated to be 16, 33, 19, 60, 81, 100, 0, 73, 52, 90, 90 and 100%, respectively. So, the qualitative data afore presented and regarded as significant relate to the bands which have more than a 50% decrease of the peak-area. Also depending on the study objective, these peak-areas can be transformed into IgG units or concentrations by relating to the concentration of total IgG antibodies eluted from bands recognized by the positive serum for toxoplasmosis. The total concentration of the eluate may be measured by sensitive assays such as immunoenzymatic (ELISA) or nephelometric.

It is interesting to note that IgG and IgM antibodies share reactivities with several *T. gondii* peptides. For instance, the 30 kDa peptide and possibly the 35 kDa peptide are both recognized by IgG and IgM antibodies.

Previous studies^{13, 16, 17, 18, 22} have shown that many antigenic bands of 6 kDa to 150 kDa are recognized by IgG antibodies from patients with chronic toxoplasmosis, most frequent among them peptides of 22-25, 30 and 35 kDa. Thus, the G-toxo-HA reagent proved to be able to detect these antibodies.

The number of antigenic bands recognized by IgM antibodies is reported^{16, 17, 18, 22} to be lower than the number of IgG antibodies, and we observed that IgM antibodies recognized bands of 32-35, 27-30, 25, 22 and 6 kDa, in particular the 35 kDa band which is considered to be the most expressive¹⁷. An immunodominant P30 antigen from the parasite membrane has been reported^{19, 20, 21} and, depending on the electrophoretic conditions, has been found in the region from 27 to 35 kDa by several investigators^{7, 10}. Thus the M-toxo-HA reagent is also capable to detect the anti P30 antibodies.

The methodology employed here permitted us to identify the major peptides involved in the G-toxo-HA and M-toxo-HA tests. IgG and IgM antibodies have been reported^{4, 12, 15} to be induced by protein and also by carbohydrate components of *T. gondii* tachyzoites. In general, IgG antibodies react with protein antigens, whereas IgM antibodies react with sugar residues from polysaccharides or glycoproteins²⁴. In the present study, carbohydrates of 32-35 kDa were found to be sensitive to m-periodate treatment, indicating the presence of glycosylated epitopes (data not shown). The M-toxo-HA reagent is prepared with the Alk-toxo antigen which is heated to 121° C for 15 min, with consequent denaturation of the protein portions. Thus, the IgM antibodies seem to be reacting mainly with sugar epitopes. In a previous study from our laboratory²⁴, eluates from the M-toxo-HA reagent contained a high proportion of IgM antibodies and a very small proportion of IgG antibodies. These IgG antibodies seem to be IgG2, which shares the same carbohydrate epitopes for IgM antibodies.

The G-toxo-HA reagent, on the other hand, contains protein epitopes and predominantly reacts with IgG antibodies. Thus, IgG antibodies react with the protein portion of the P30 antigen molecule found in G-toxo-HA reagent, whereas IgM antibodies react with the carbohydrate portion of the same antigen also found in the M-toxo-HA reagent.

Further studies are required to confirm the present findings, with a larger number of sera from toxoplasmosis patients with high, medium and low IgG and IgM antibody titers. Sera with low antibody titers were not studied here, but it is assumed that they will be able to react with epitopes of the 30 kDa band because this component has been reported to be highly immunogenic and to promote antibody production at an early stage of *T. gondii* infection^{20, 21}.

The two processes studied here of antibody absorption and antibody elution indirectly reveal the actual antigen epitopes present in the G-toxo-HA and M-toxo-HA reagents. These findings, taken together with those reported by LIM¹¹ suggest that antibody elution is more practical than antibody absorption. High antibody levels require more than one absorption step, making the latter process cumbersome.

The methodology here employed will be helpful for the study of other serologic tests and may provide valuable information about antigenic components relevant to the immunodiagnosis, prognosis or therapeutic control of different parasitic infections.

RESUMO

Identificação de antígenos de *Toxoplasma gondii* envolvidos nas reações de hemaglutinação passiva IgM e IgG destinadas ao sorodiagnóstico da toxoplasmose

Extratos brutos de *Toxoplasma gondii* constituem matéria prima antigênica para o preparo de reagentes empregados em diferentes testes sorológicos para o diagnóstico da toxoplasmose, incluindo entre estes as reações de hemaglutinação indireta para a detecção de anticorpos IgM (HA IgM) e IgG (HA IgG). Até o presente momento, moléculas antigênicas do parasita que realmente estão envolvidas na interação com anticorpos aglutinantes, anti-*T. gondii*, não são ainda bem conhecidas. O processo de absorção de soros de pacientes com toxoplasmose, utilizando o reagente de HA IgG (HA-toxo-G), possibilitou a demonstração de que hemácias deste reagente estavam sensibilizadas com antígeno do parasita associado às bandas de massa molecular relativa de 39, 35, 30, 27, 22 e 14 kDa. O processo de imunoabsorção com o reagente de HA IgM (HA-toxo-M) por sua vez forneceu eluatos de anticorpos, que reconheceram bandas antigênicas correspondendo a 54, 35 e 30 kDa, indicando a existência destes antígenos ligados às hemácias do reagente.

A identificação de antígenos mais relevantes para cada tipo de reagente de hemaglutinação poderá prestar auxílio na inspeção da matéria prima antigênica, assim como das partidas de reagentes produzidas rotineiramente. Ademais, os achados fornecem subsídios para introduzir novas modificações nestes reagentes, objetivando a melhoria no desempenho dos testes de HA para o diagnóstico da toxoplasmose.

ACKNOWLEDGEMENTS

We wish to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - proc. 87/1991-4) and Conselho

Nacional de Desenvolvimento Científico e Tecnológico (CNPq - proc. n° 301233/96-9) and PIBIC-USP/CNPq for financial support.

REFERENCES

1. AUSUBEL, F.M.; BRENT, R.; KINGSTON, R.E. et al. - Current protocols in molecular biology. New York, John Wiley & Sons, 1989. p. 10.2.1. - 10.8.5.
2. CAMARGO, M.E.; LESER, P.G. & ROCCA, A. - Definições de perfis sorológicos na toxoplasmose. Importância diagnóstica e epidemiológica. **Rev. bras. Pat. clin.**, 13: 113-127, 1977.
3. CAMARGO, M.E.; HOSHINO, S. & SIQUEIRA, G.R.V. - Hemagglutination with preserved, sensitized cells, a practical test for routine serologic diagnosis of American trypanosomiasis. **Rev. Inst. Med. trop. S. Paulo**, 15: 81-85, 1973.
4. CAMARGO, M.E.; FERREIRA, A.W.; MINEO, J.R.; TAKIGUTI, C.K. & NAKAHARA, O.S. - Immunoglobulin G and immunoglobulin M enzyme linked immunosorbent assays and defined toxoplasmosis serological patterns. **Infect. Immun.**, 21: 55-58, 1978.
5. CAMARGO, M.E.; MOURA, M.E.G. & LESER, P.G. - Toxoplasmosis serology: an efficient hemagglutination procedure to detect IgG and IgM antibodies. **Rev. Inst. Med. trop. S. Paulo**, 31: 279-285, 1989.
6. CHUMPITAZI, B.F.F.; BOUSSAID, A.; PELLOUX, H. et al. - Diagnosis of congenital toxoplasmosis by immunoblotting and relationship with other methods. **J. clin. Microbiol.**, 33: 1479-1485, 1995.
7. COUVREUR, G.; SADAK, A.; FORTIER, B. & DUBREMETZ, J.F. - Surface antigens of *Toxoplasma gondii*. **Parasitology**, 97: 1-10, 1988.
8. FUCHS, S & SELA, M. - Immunoabsorbants. In: WEIR, D.M. - Handbook of experimental immunology. Immunochemistry. 4. ed. Oxford, Blackwell, 1986. v.1.p.16. 1-16.5
9. HUGHES, H.P.A. & BALFOUR, A.H. - An investigation of the antigenic structure of *Toxoplasma gondii*. **Paras. Immunol.**, 31: 235-248, 1981.
10. KASPER, L.H.; CRABB, J.H. & PFEFFERKORN, E.R. - Purification of a major membrane protein of *Toxoplasma gondii* by immunoabsorption with a monoclonal antibody. **J. Immunol.**, 130: 2407-2412, 1983.
11. LIM, P.L. - Isolation of specific IgM monoclonal antibodies by affinity chromatography using alkaline buffers. **Molec. Immunol.**, 24: 11-15, 1987.
12. MINEO, J.R.; CAMARGO, M.E. & FERREIRA, A.W. - Enzyme linked immunosorbent assay for antibodies to *Toxoplasma gondii* polysaccharides in human toxoplasmosis. **Infect. Immun.**, 27: 283-287, 1980.
13. MOIR, I.L.; DAVIDSON, M.M. & HO-YEN, D.O. - Comparison of IgG antibody profiles by immunoblotting in patients with acute and previous *Toxoplasma gondii* infection. **J. clin. Path.**, 44: 569-572, 1991.
14. NAKAHARA, O.S.; HOSHINO-SHIMIZU, S. & YAMAMOTO, Y.I. - Evaluation of an enzyme immunoassay (IgM capt dot EIA) using an enzyme labelled *Toxoplasma gondii* antigen (alk-Toxo) for the diagnosis of acute toxoplasmosis. **Serodiag. Immunother. infect. Dis.**, 7:40-44, 1995.
15. NAOT, Y.; GUPTILL, D.R.; MULLENAX, J. & REMINGTON, J.S. - Characterization of *Toxoplasma gondii* antigens that react with human immunoglobulin M and immunoglobulin G antibodies. **Infect. Immun.**, 41: 331-338, 1983.
16. PARTANEN, P.; TURUNEN, H.J.; PAASIVUO, R.T.A. et al. - Identification of antigenic components of *Toxoplasma gondii* by an immunoblotting technique. **FEBS Lett.**, 158: 252-254, 1983.
17. PARTANEN, P.; TURUNEN, H.J.; PAASIVUO, R.T.A. & LEINIKKI, P.O. - Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulins G, M and A antibodies at different stages of infection. **J. clin. Microbiol.**, 20: 133-135, 1984.
18. POTASMAN, I.; ARAÚJO, F.G.; DESMONTS, G. & REMINGTON, J.S. - Analysis of *Toxoplasma gondii* antigens recognized by human sera obtained before and after acute infection. **J. infect. Dis.**, 154: 650-657, 1986.
19. RODRIGUEZ, C.; AFCHAIN, D.; CAPRON, A.; DISSOUS, C. & SANTORO, F. - Major surface protein of *Toxoplasma gondii* contains an immunodominant region with repetitive epitopes. **Europ. J. Immunol.**, 15: 747-749, 1985.
20. SANTORO, F.; AFCHAIN, D.; PIERCE, R. et al. - Serodiagnosis of toxoplasma infection using a purified parasite proteins (p 30). **Clin. exp. Immunol.**, 62: 262-269, 1985.
21. SANTORO, F.; CHARIF, H. & CAPRON, A. - The immunodominant epitope of the major membrane tachyzoite protein (p 30) of *Toxoplasma gondii*. **Paras. Immunol.**, 8: 631-635, 1986.
22. SHARMA, S.D.; MULLENAX, J.; ARAÚJO, F.G.; EHRLICH, H.A. & REMINGTON, J.S. - Western blot analysis of the antigen of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. **J. Immunol.**, 131: 977-983, 1983.
23. SUZUKI, Y.; THULLIEZ, P.; DESMONTS, G. & REMINGTON, J.S. - Antigen(s) responsible for immunoglobulin G responses specific for the acute stage of toxoplasma infection in humans. **J. clin. Microbiol.**, 26: 901-905, 1988.
24. YAMAMOTO, Y.I. - Toxoplasmose aguda: desenvolvimento do reagente de hemaglutinação passiva (HA-toxo-IgM) para fins de diagnóstico. São Paulo, 1987. (Tese de Doutorado - Instituto de Ciências Biomédicas da Universidade de São Paulo).
25. YAMAMOTO, Y.I.; HOSHINO-SHIMIZU, S. & CAMARGO, M.E. - A novel IgM-indirect hemagglutination test for the serodiagnosis of acute toxoplasmosis. **J. clin. Lab. Anal.** 5: 127-132, 1991.

Recebido para publicação em 19/05/97
Aceito para publicação em 14/08/97