Rev. Inst. Med. trop. S. Paulo
39(4): 235-236, July/August, 1997

BRIEF COMMUNICATION

DOT-ENZYME-LINKED IMMUNOSORBENT ASSAY (DOT-ELISA) FOR EVALUATING IgG ANTIBODY AVIDITY IN TOXOPLASMOSIS

Fabio G. MELO & Claudio L. ROSSI

KEYWORDS: Toxoplasmosis; Antibody avidity; Dot-ELISA.

The detection of *Toxoplasma*-specific IgM antibodies has been considered one of the most valuable tools for diagnosing acute toxoplasmosis. However, in some patients, IgM antibodies can be detected for a very long time after the acute phase of infection[1,3], particularly when more sensitive techniques are used. The low avidity of IgG antibodies in recent primary infections has become a useful tool for improving serological diagnosis of many infections, including toxoplasmosis[2,4,6]. Avidity tests are generally based on immunological methods in which a denaturing agent, such as urea, is used to dissociate the low-avidity antibodies after the antigen-antibody interaction[2,4,5,6]. This report describes a simple and rapid avidity test for toxoplasmosis using a Dot-enzyme-linked immunosorbent assay (Dot-ELISA).

Unless otherwise stated, the chemicals and reagents used in this study were obtained from Sigma Chemical Company (St. Louis, MO, USA).

The *Toxoplasma* antigen was prepared as previously described[8] with a few modifications. Briefly, tachyzoites of the N strain of *T. gondii*[7], obtained from the peritoneal cavity of Swiss mice two days after infection, were washed four times by centrifugation in 0.15 M phosphate-buffered saline (PBS), pH 7.2. The final parasite pellet was resuspended in PBS to about ten times the pellet volume and the suspension then sonicated for two minutes in an ice water bath using a Branson sonicator (model SX-30B, Branson Ultrasonics, Danbury, USA) at a power setting of three with a 20% pulse duty cycle. One microliter each of 0.25 M phenylmethylsulphonyl fluoride and 0.1% leupeptin solutions were added to each milliliter of sonicated material and the suspension was gently stirred for 18 hours at 5°C. After centrifugation at 18,000 × g for 30 minutes at 5°C, the supernatant (final antigen) was carefully removed, and stored at – 70°C until used.

A nitrocellulose sheet (8.5 × 6 cm; pore size, 0.2-µm; Schleicher & Schuell, USA) was placed on a plastic sheet and then cut into strips 4 mm wide using a homemade strip cutter. The *Toxoplasma* antigen at 32 µg/ml in PBS was applied to the nitrocellulose using a precision pipet; one dot of one microliter was placed on each strip. After antigen application, the strips were air dried at room temperature for 30 minutes. All subsequent reactions were performed at room temperature in the troughs of incubation trays (Schleicher & Schuell, USA) containing 0.5 ml of the reagents. During the incubation and washing steps, the trays were placed on a rocker and gently rocked to ensure total submersion of the strips. Sensitized strips were transferred to two multichannel trays which had an identical range of serum dilutions in PBS containing 0.3% Tween 20 (PBS/T) and 5% non-fat milk (serum titrations ranged from 1/20 to 1/20,480). After incubation for 5 minutes, the unbound serum components were removed by three washes (2 min each) with PBS/T. After washing, the troughs of one plate were filled with PBS and those of the other with PBS containing 6 M urea. After incubation and washing as previously described, all the strips were incubated for 5 minutes with conjugate (goat anti-human IgG – peroxidase) diluted 1:250 in PBS/T. Following three washes (2 min each) with PBS/T, the bound antibodies were visualized by incubating the strips for 5 minutes in a diaminobenzidine-hydrogen peroxide substrate system. After ten washes (1.5 min each) with distilled water to stop the reaction, the strips were removed from the trays, placed on a plastic sheet and air dried. The reactions were visually read;

Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, São Paulo, Brasil.
Correspondence to: Claudio L. Rossi. Departamento de Patologia Clínica. Faculdade de Ciências Médicas, Universidade Estadual de Campinas, C.P. 6111, 13083-970 Campinas SP, Brasil.

strips showing clearly defined brown dots were considered positive. Two antibody titers were obtained for each tested serum sample, one for the urea-treated strips (PBS/urea titer) and the other for strips not exposed to urea (PBS titer). The PBS and PBS/urea antibody titers were given as the highest serum dilution showing a positive reaction. Low-and high-avidity antibodies are predominantly found in recent and long-term infections, respectively. A fourfold or greater decrease in the PBS/urea titer was considered as indicative of a recent toxoplasmic infection.

Two groups of serum samples were assayed by the Dot-ELISA: Group I consisted of five serum samples taken within the first two months after the onset of toxoplasmic lymphadenopathy (TL). Group II consisted of five serum samples taken twelve or more months after the onset of TL. Three of the latter serum samples exhibited significant levels of *Toxoplasma*-specific IgM antibodies which were detected by an indirect immunofluorescence (IIF) test and/or by an antibody capture ELISA (cELISA) technique. The five serum samples collected during the early phase of toxoplasmic infection presented a significant decrease in antibody titers (≥16-fold) after urea treatment, a result compatible with the predominance of low-avidity antibodies. On the other hand, high-avidity antibodies were predominant in the five serum samples obtained twelve or more months after the onset of clinical symptoms of infection as indicated by the unaltered antibody titers after urea treatment (Table 1).

The Dot-ELISA described here was able to distinguish recent from long-term *Toxoplasma* infections. The test can be performed rapidly without the use of sophisticated equipment. An entire assay can be accomplished in approximately two hours with the only apparatus required being a rocker.

TABLE 1

Serological results (IgM-IIF and IgM-cELISA) and avidity tests for serum samples collected at different times after *T. gondii* infection.

Serum sample	Time*	Antibody test results IgM-IIF	IgM-cELISA	Avidity test titers PBS	PBS/urea
Group I					
1	1	2,048	R	320	20
2	2	1,024	R	1,280	80
3	2	1,024	R	2,560	80
4	2	1,024	R	2,560	160
5	2	512	R	2,560	160
Group II					
6	12	< 32	NR	10,240	10,240
7	13	64	R	5,120	5,120
8	13	64	R	1,280	640
9	14	< 32	R	2,560	2,560
10	15	< 32	NR	2,560	2,560

* Months from the onset of clinical lymphadenopathy to the time of blood sampling. Significant antibody levels are defined as IgM-IIF ³ 32 and IgM-cELISA = R (R = Reactive; NR = Non-reactive). Avidity test: two antibody titers were obtained for each tested serum sample, one for the urea-treated strips (PBS/urea titer) and the other for strips not exposed to urea (PBS titer). A fourfold or greater decrease in the PBS/urea titer was considered as indicative of a recent toxoplasmic infection (predominance of low-avidity antibodies).

REFERENCES

1. BROOKS, R.G.; McCABE, R.E. & REMINGTON, J.S. – Role of serology in the diagnosis of toxoplasmic lymphadenopathy. **Rev. infect. Dis., 9**:1055-1062, 1987.

2. CAMARGO, M.E.; DA SILVA, S.M.; LESER, P.G. et al. – Avidez de anticorpos IgG específicos como marcadores de infecção primária recente pelo *Toxoplasma gondii*. **Rev. Inst. Med. trop. S. Paulo, 33**:213-218, 1991.

3. DEL BONO, V.; CANESSA, A.; BRUZZI, P. et al. – Significance of specific immunoglobulin M in the chronological diagnosis of 38 cases of toxoplasmic lymphadenopathy. **J. clin. Microbiol, 27:**2133-2135, 1989.

4. HEDMAN, K.; LAPPALAINEN, M.; SEPPALA, I. et al. – Recent primary *Toxoplasma* infection indicated by a low avidity of specific IgG. **J. infect. Dis., 159**:736-740, 1989.

5. HEDMAN, K. & SEPPALA, I. – Recent rubella virus infection indicated by a low avidity of specific IgG. **J. clin. Immunol., 8**:214-221, 1988.

6. HOLLIMAN, R.E.; RAYMOND, R.; RENTON, N. et al. – The diagnosis of toxoplasmosis using IgG avidity. **Epidem. Infect., 112**:399-408, 1994.

7. NÓBREGA, P.; TRAPP, L.E. & GIOVANNONI, M. – Toxoplasmose epizoótica em coelhos. I. Ação de sulfadiazina. **Ciênc. e Cult., 4**:134-135, 1952.

8. TAKAHASHI, E.E.H. & ROSSI, C.L. – Use of three immunological techniques for the detection of *Toxoplasma* sp IgA antibodies in acute toxoplasmosis. **J. clin. Path., 47**:1101-1104, 1994.

Recebido para publicação em 28/04/1997
Aceito para publicação em 28/08/1997