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ESTABLISHMENT OF IMMUNOGLOBULIN M(IgM)-IMMUNOSORBENT AGGLUTINATION ASSAY (ISAGA) FOR DIAGNOSIS OF TOXOPLASMOSIS

ZHANG Shuyi, WEI Meixiong, ZHAO Huifen, SHI Gongfen
Shanghai Detecting Center for Toxoplasmosis
Shanghai Institute of Parasitic Disease Control and Rsesarch, Shanghai 200336

ABSTRACT

AIM: To establish an immunosorbent agglutination assay (ISAGA) for detection of IgM antibodies against *Toxoplasma gondii*. **METHODS**: In the ISAGA, wells of microtiter plates were coated with anti-human IgM antibodies and sealed with 1% bovine serum albumin. After the test sera were added and incubated, the plates were washed, *T. gondii* tachyzoite antigen suspension was added, and incubated overnight at 37 ℃. The ISAGA results were evaluated by comparing with those detected by Danish ISAGA and ELISA and those detected by slide enzyme immunoassay (S-EIA). **RESULTS**: Forty-four sera from Danish pregnant women were tested by the IgM-ISAGA, 41 (93.2%) were consistent with the Danish results. Sixty-seven sera from Danish and Shanghai pregnant women were detected by IgM-IgM-ISAGA and S-EIA, the total consistency rate was 92.5%. A significant correlation was found between the titers of the ISAGA and S-EIA, with a Pearson correlation coefficient of 0.589 ($P < 0.001$). The titers of ISAGA were eighteen times higher than those of S-EIA. This method enables the detection of IgM antibodies as low as approximately 0.08 IU/ml. **CONCLUSION**: The IgM-ISAGA is therefore sensitive, specific, easy to perform, and is useful for mass screening and diagnosing recent *Toxoplasma* infection or reactivation.

Key words: *Toxoplasma gondii*, immunodiagnosis, IgM-ISAGA

Toxoplasma gondii is a protozoan parasite of worldwide distribution that causes both animal and human toxoplasmosis. Diagnosis of toxoplasmosis is routinely based on serological tests because detection by histological examinations of biopsy specimens or by isolation of *T. gondii* from body fluids or tissues is rather difficult and impractical. Since the introduction of the dye test by Sabin and Feldman (1948) for detecting *Toxoplasma* antibodies, many serological techniques including the complement fixation, indirect hemagglutination (IHA), indirect immuno-fluorescence antibody (IFA) tests and enzyme-linked immunosorbent assay (ELISA) have been used. The detection of *Toxoplasma* specific IgM by IFA and ELISA is valuable for rapid diagnosis of acute congenital and acquired toxoplasmosis. Recently, an immunosorbent agglutination assay (ISAGA) has been introduced for the determination of IgM antibodies against *Toxoplama*[1～3] and the ISAGA commercial kit is now available[4～6]. The aim of the present study was to establish an ISAGA for detecting *Toxoplasma* specific IgM antibodies.

MATERIALS AND METHODS

Preparation of antigen

Tachyzoites of the RH strain of *Toxoplasma gondii* were purified and fixed as previously described for the direct agglutination test[8]. Briefly, the tachyzoites obtained from peritoneal exudates of mice infected for 3 days were washed with PBS (pH7.2) and treated with 0.25% trypsin (Difco, USA) in PBS for 30 min at 37 ℃, and then washed five times as described above. After centrifugation, the parasites were suspended in 6% formaldehyde solution and kept overnight at 4 ℃. Following fixation, the parasites were washed three times in PBS and resuspended in an alkaline buffer at a concentration of 2×10^7 organisms per ml. The suspension of antigen was stored at 4 ℃ until used.

Anti-IgM serum

A goat antiserum to purified human IgM (Shanghai Institute of Biological Products) was purified by 33% ammonium sulfate precipitation and DEAE-52 cellulose (Whatman, UK) and stored at -80 ℃ until used.

Human serum samples

Forty-four sera from Danish pregnant women, 33 sera from Shanghai pregnant women, and 1 international standard for human anti-*Toxoplasma* serum, 2 000 international units per ampoule, WHO International Laboratory for Biological Standards.

IgM-ISAGA

The assay was conducted as follows: the wells of

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U-shaped microtiter plates (Nunc, A/S, Inter Med, Denmark) were first coated with 100 μl of the anti-human IgM diluted in 0.05 mol/L carbonate buffer(pH9.6). The plates were stored overnight at 4 ℃ or incubated for 2 h at 37 ℃, and washed three times for 5 min each with PBS containing 0.05 % Tween-20 (PBST). The wells were then sealed with 100 μl of 1 % bovine serum albumin in PBS and stored at 4 ℃ until used. After the plates were washed, 100 μl of each serum sample diluted serially from 1∶20 in PBST was added to the wells, and the plates were incubated at 37 ℃ for 2 h. After the plates were washed as mentioned above, 50 μl of the suspension of antigen was added to the wells and incubated overnight at 37 ℃. In each test, a positive control and a negative control were included. The pattern of agglutination was read as previously described.[8] The ISAGA titers ≥1∶20 for IgM were regarded as positive.

The optimal concentrations of anti-IgM antibodies to be used for the ISAGA was determined by checkerboard titration using the international standard serum as the positive control and a pool offive sera obtained from healthy newborn infants as the negative control. The results of effects of various concentrations of anti-IgM antibodies by ISAGA showed that at lower concentrations of anti-IgM antibodies false-negative results might occur in sera containing low concentrations of IgM antibodies against *Toxoplasma*. To attain a high degree of specificity and sensitivity, a concentration of anti-IgM antibodies of 30 μg～50 μg per ml seemed to be optimal for the subsequent detection.

Reference tests

①Danish tests (IgM-ISAGA and IgM-ELISA) were performed by the laboratory of Professor Eskild Pertersen, Statens Seruminstitut, Denmark. ②Slide enzyme immunoassay (S-EIA) was performed in this center. In the S-EIA, forrmalin-fixed tachyzoites were dropped onto grease-free clean slides, air-dried in a refrigerator and stored at 4 ℃ until needed. Serum samples were tested with two-fold dilutions starting from 1∶5. A horseradish peroxidase-labeled rabbit anti-human IgM conjugate (Sino-American Biotechnology Co. Shanghai Branch) was used at a dilution of 1∶20. The S-EIA titers ≥1∶5 for IgM were regarded as positive.

RESULTS

Among forty-four Danish pregnant women sera tested in this Center, 27 (61.4 %) were positive and 17 were negative by ISAGA, whereas in the laboratory of Professor Eskild Petersen, 30 (68.2 %) samples were positive and 14 samples were negative by Danish tests. When compared our results with Danish results, the positive consistency rate was 100.0 %(27/27), the negative consistency rate was 82.4 %(14/17), and the total consistency rate was 93.2 %(41/44).

Serum samples from thirty-four Danish pregnant women and thirty-three Shanghai pregnant women were examined by ISAGA and slide EIA. The results showed that the positive consistency rate was 85.2 % (23/27). The two tests disagreed in 7.5 % (5/67) of the samples. The relative titers of the ISAGA compared with S-EIA for 67 sera are shown in Table 3. Of the 40 sera found negative by ISAGA, 39 (97.5 %) were also negative by S-EIA. Four sera were positive by the ISAGA but gave negative results in S-EIA. As shown in, a significant correlation was found between the titers determined by ISAGA and S-EIA, with the Pearson correlation coefficient of 0.589 ($P < 0.001$), and the ISAGA titers were eighteen times higher than S-EIA titers.

DISCUSSION

The *Toxoplasma* IgM-ISAGA is a novel technique developed in the 1980s, which was first described by Desmonts et al[1]. Desmonts[2] reported that IgM antibodies were detected in 76 % of cord sera or sera taken from the newborn baby with a congenital *Toxoplasma* infection (whlie 25 % only were positive in the IgM-IFA), and positive results in the ISAGA were also obtained in more than 90 % of sera taken from patients with a recent acquired infection. Desmonts and Thulliez[3] introduced the ISAGA for routine screening and diagnosing toxoplasmal infection in the mother and infant in France. Hlobil et al[9] used ISAGA for the screening of *Toxoplasma* infections in pregnant women in Germany.

Recently, the IgM-ISAGA has become one of the most satisfactory methods available for the determination of IgM antibodies against and for the diagnosis of acute congenital and acquired infection with *T. gondii* in Western countries. A collaborative, multi-center study designed to evaluate the different techniques for the diagnosis of *Toxoplasma* infection, involving 129 European laboratories, showed that ISAGA proved to be the most sensitive method for the detection of specific IgM[4].

IgM-ISAGA developed in this center was evaluated by comparing it with Danish tests (ISAGA and ELISA)

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and S-EIA. The results showed that high consistency was qualitatively shown between the ISAGA and Danish tests (93.2 %) and between the ISAGA and S-EIA (92.5 %), respectively. The ISAGA was proved to have high sensitivity and specificity. With the results obtained by Danish tests as standard the sensitivity and specificity of ISAGA were found to be 90 % and 100 %, respectively. A significant correlation was found between the titers of the ISAGA and S-EIA, with a Pearson correlation coefficient of 0.589 ($P < 0.001$). The titers of the ISAGA were shown to be eighteen times higher than those of S-EIA. The ISAGA results of eight detections with the WHO international standard for *Toxoplasma* antibodies (1 000 IU/ml) showed that positive dilution of 1∶10 240 in 6 detections and 1∶20 480 in 2 detections, and the geome tric mean titer was 1∶12 180 which corresponds to a concentration of 0.08 IU/ml.

The principle of ISAGA is based on a combination of antibody capture method and agglutination test, therefore it technologically shares the advantages of immunosorbent assay and agglutination test. Compared with the conventional ELISA, can be performed and read without special equipment and an enzyme-labeled anti-human IgM conjugate and substrate in development system. So the ISAGA is simpler, easier and more rapid to perform than ELISA. Thus, the ISAGA proved to be useful for mass screening and diagnosing recent *Toxoplasma* infection or reactivation.

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弓形虫病 IgM 免疫吸附凝集试验 (ISAGA) 的建立

张述义　魏梅雄　赵惠芬　石恭芬
上海市寄生虫病防治研究所　上海市弓形虫病检测中心　上海　200336

提要　目的:建立检测弓形虫 IgM 抗体的免疫吸附凝集试验 (ISAGA)。**方法**:U 型微孔板以适宜浓度的羊抗人 IgM 抗体包被,用 1 %牛血清白蛋白封闭,洗板后加入待测血清,在 37 ℃孵育后洗涤,加入弓形虫 (RH 株) 速殖子抗原悬液,置 37 ℃过夜后观察结果。将其与丹麦的 ISAGA 和 ELISA 检测的结果以及玻片 EIA 检测的结果作比较。**结果**:本法与丹麦的试验检测丹麦孕妇 44 份血清,总符合率为 93.2 %;与玻片 EIA 检测丹麦和上海孕妇 67 份血清,总符合率为 92.5 %,两法的滴度之间明显相关 ($\gamma = 0.589$, $P < 0.001$),ISAGA 滴度较玻片 EIA 高 18 倍;用 WHO 国际生物标准化实验室提供的标准的抗弓形虫人血清定量测得其灵敏度为 0.08 IU/ml。**结论**:IgM-ISAGA 具有敏感性、特异性高,操作简便等优点,不仅可作为弓形虫急性感染和慢性感染活动期的一种检测手段,而且适用于弓形虫感染调查的大规模筛选。

关键词　弓形虫　免疫诊断　IgM-ISAGA

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