Assessment of immunoglobulin-M immunosorbent agglutination assay (ISAGA) for detecting toxoplasma specific IgM

KIM T DUFFY, P J WHARTON, JULIE D JOHNSON, LINDA NEW, R E HOLLIMAN
Public Health Laboratory Service, Toxoplasma Reference Laboratory, St George's Hospital, London

SUMMARY An immunoglobulin-M immunosorbent agglutination assay (ISAGA) was introduced to detect toxoplasma specific IgM. This assay incorporates μ chain capture and use of entire toxoplasma trophozoites as an antigen source. The performance of the ISAGA was compared with that of a double sandwich enzyme linked immunosorbent assay (DS-ELISA) currently used in the Public Health Laboratory Service Toxoplasma Reference Laboratories. The ISAGA was found to be more sensitive than DS-ELISA but there was no demonstrable difference in the specificity or reproducibility between the two assays.

The ISAGA is suitable for the diagnosis of acute toxoplasmosis in immunocompetent patients and as a screening test for recent infection in pregnant women. The persistence of ISAGA reactivity, however, is such that additional serological assessment is required to define the risk of congenital infection.

Acute toxoplasma infection in immunocompetent patients is usually asymptomatic. The few who develop a clinical illness present with non-specific signs and symptoms such as malaise and lymphadenopathy.¹ In clinical practice the diagnosis of toxoplasmosis is based on serological findings. Measurement of specific IgM antibody is of known value,² and several detection methods have been developed, including the indirect immunofluorescent antibody test (IFAb),³ latex agglutination assay,⁴ and the enzyme linked immunosorbent assay (ELISA).⁵ The double sandwich enzyme linked immunosorbent assay (DS-ELISA) has been shown to be more sensitive than IFAb or conventional ELISA methods.⁶ Furthermore, the initial μ chain capture reduces the incidence of false negative reactions associated with the presence of excess toxoplasma IgG and false positive results due to rheumatoid factor or antinuclear antibody which are known hazards of the conventional ELISA or IFAb test.⁷

The IgM latex agglutination assay, IFAb test, ELISA and DS-ELISA use disrupted toxoplasma organisms as an antigen source. French workers have proposed that toxoplasma serology tests should use whole toxoplasma trophozoites as antigens.⁸ They suggest that the presentation of the organism in its

entirety is an accurate reflection of the antigenic stimulation of the immune system induced in acute infection. Consequently, assays based on whole toxoplasma trophozoites may prove superior for the detection of the human antibody response in toxoplasmosis. The immunoglobulin-M immunosorbent agglutination assay (ISAGA) is routinely used in France for the diagnosis of acute toxoplasmosis and congenital infection.⁸ This assay uses a monoclonal antibody to the CH2 domain of the human μ chain, allowing IgM capture, while avoiding cross reactivity with IgG, IgA, or IgE.⁹ Specific IgM is then shown by the agglutination of whole toxoplasma trophozoites.¹⁰ As the ISAGA has recently become available in this country we undertook a study to compare the performance of this assay with that of the DS-ELISA currently used in our laboratory for detection of toxoplasma specific IgM.

Material and methods

The immunosorbent agglutination assay (ISAGA) detects human IgM antibodies to toxoplasma. An anti-human IgM monoclonal antibody is used to capture human IgM in serum or cerebrospinal fluid. The addition of toxoplasma trophozoites shows the presence of specific toxoplasma IgM.

The kit consists of a 4 ml suspension of formalin treated *Toxoplasma gondii* trophozoites obtained

from mouse ascitic fluid, 80 ml of diluent (BABS colour buffer), 0.5 ml positive control sera, powdered phosphate buffered saline (PBS) sufficient for 2 litres, and 1 ml of Tween 20. Also included are four report sheets and four sachets each containing six strips of 2×8 round base wells, sensitised with anti-human IgM monoclonal antibody, presented in a microtitre format. The shelf life of this ISAGA kit is said to be six months at 4°C.

The manufacturers suggest a screening procedure followed by a confirmatory test performed only on positive samples. In this trial a single assay was performed. Each serum sample was placed in three wells to which were added 100, 150, or 200 µl of antigen suspension. Sera were diluted at 1/100 in PBS, except those for neonates which were diluted at 1/20. The positive control serum was diluted at 1/10. PBS alone was used as a negative control. Each serum dilution (100 µl) was placed into three adjacent wells. The strips were then covered and incubated at 37°C in a moist chamber for two hours. After incubation the wells were emptied by inverting the plate. Each well was washed twice in PBS-Tween and once in PBS alone. The plate was drained thoroughly by tapping on clean paper towels. The toxoplasma antigen was diluted to 1/20 with BABS buffer and added to the wells, as previously described. The strips were then covered, placed in a moist chamber, and left at 37° overnight. Agglutination patterns were determined using a mirrored plate reader.

A negative reaction resulted in sedimentation of the toxoplasma trophozoites forming a compact button. The results were marked on a scale of 0 to 4. Total sedimentation in a button similar to the antigen (negative) control, with no agglutination, was given a value of 0, a large button a value of 1, medium sized button 2, very small button 3, and no visible sediment 4. Each well was scored and the total for the three wells was called the "ISAGA index". The ISAGA index was interpreted as follows: 0 to 5, negative reaction; 6 to 8, borderline; 9 to 12, positive reaction.

Methods for the DS-ELISA,¹¹ the dye test (DT),¹² latex agglutination test (LAT),¹³ and direct agglutination test (DAT)¹⁴ were used as previously described. Toxoplasma specific IgM measurements were performed on routine serum samples producing a DT result of > 31 units, or when the DAT or LAT findings were suggestive of acute infection. ISAGA and DS-ELISA were also performed on samples from patients who were human immunodeficiency virus (HIV) positive, transplant recipients or who suffered from retinochoroiditis, and mother and baby samples in cases of possible congenital infection.

The results of the ISAGA and DS-ELISA were defined in the following way: if both assays were positive IgM was positive; if both were negative IgM

was negative. Where one assay gave positive results and the other negative these were termed "mismatches". Both assays were then repeated. Where one assay produced a borderline or equivocal result and the other a clear positive or negative result, these were not regarded as "mismatches".

The sensitivity, specificity, and reproducibility of the tests were assessed using separate selected sera. Five sera showing high DS-ELISA results (300–400 enzyme immunoassay units (EIU)) were diluted sequentially in physiological saline to produce a 10% dilution sequence and each dilution tested for specific IgM using ISAGA and DS-ELISA. A selection of sera with the potential to produce false positive IgM reactions were tested by ISAGA and DS-ELISA. Five sera, each positive for antinuclear factor, cytomegalovirus specific IgM, rheumatoid factor, hepatitis A specific IgM, or Epstein-Barr virus specific IgM were processed. Five jaundiced sera, five haemolysed sera, and five sera known to produce negative dye test and latex reactions were also tested. The negative sera were subsequently heat inactivated at 56°C for three hours and retested. Fifty sera previously found to have no detectable toxoplasma specific antibody by dye test or latex agglutination were assayed. Potential false negative reactions were investigated by selecting five sera producing positive DS-ELISA and ISAGA results. Each sample was freeze thawed five times and then retested for specific IgM. The reproducibility of the two assays was assessed by the selection of 10 sera representing a range of IgM DS-ELISA results from negative to high positive (0–400 EIU). Each sample was assayed by DS-ELISA and ISAGA five times. When a patient's serum was found to give a positive reaction by both IgM assays, repeated samples were requested to document the decline of IgM concentrations by the two tests.

The cost, ease of performance, equipment required and MLSO time necessary for ISAGA and DS-ELISA performance were assessed during the course of the study.

Results

A total of 1313 routine samples were processed (table). Of the sera tested, 1032 (79%) samples produced negative results in both the ISAGA and DS-ELISA. Of 145 sera which gave a positive DS-ELISA result, 140 (97%) were confirmed by ISAGA. Of the 222 samples which gave an ISAGA positive result, however, 70 (32%) were negative when tested by DS-ELISA. There were 72 confirmed mismatch results; of these, 70 were DS-ELISA negative/ISAGA positive and only two were DS-ELISA positive/ISAGA negative. The results of 30 samples were designated as "test errors" (defined as changing from positive to

Table Comparison of DS-ELISA and ISAGA results

	DS-ELISA Result			Total
	Positive	Borderline	Negative	
ISAGA results:				
Positive	140	12	70	222
Borderline	3	14	39	56
Negative	2	1	1032	1035
Total	145	27	1141	1313

negative, or vice versa, by either assay on repeat testing). Review of these results showed that 11 errors occurred in the DS-ELISA, 18 errors in the ISAGA, and one sample produced technical errors in both tests. Of five sera tested in dilution from 90% to 10%, for three samples the ISAGA result remained positive at a dilution higher than that of the DS-ELISA. In no instance did the DS-ELISA remain positive at a higher dilution than that of the ISAGA. No false positive or false negative reactions were recorded in a range of so called "problem" sera.

Both tests were equally reproducible. Using the *f*-ratio test the variance of the DS-ELISA results was not greater than the variance of the ISAGA findings. The DS-ELISA test entailed testing four dilutions of the three control sera. If the results of these four dilutions differed by more than 10% then the test was repeated. If this rule was strictly adhered to then $\approx$ 30% of the DS-ELISA test would have to be repeated. An ISAGA test run had to be repeated when the positive control read less than nine or the negative control produced a positive result. In no instance did the tests need repeating for these reasons. Out of 14 kits used, however, four strips seemed to be defective. If there was obvious discrepancy between the three ISAGA wells used for any one sample it was repeated. This occurred in 1% of tests performed.

There were 16 instances where a series of sera were received on a particular patient. The initial serum result only was included in the figures in the table. Of these 16 series, nine showed loss of DS-ELISA reactivity first and four series showed loss of ISAGA reactivity at a time when DS-ELISA reactivity persisted.

The cost of performing the ISAGA test was about £1.30 a sample. Used as a screening test, as described by the manufacturer, the cost would be 90p a sample. The DS-ELISA is not available commercially and is produced "in house" at the Toxoplasma Reference Laboratory, Swansea. There are, however, several similar μ chain capture ELISA assays available on a commercial basis. The ISAGA test was found to be technically easier to perform than the DS-ELISA. It required only a mirrored plate reader as additional equipment. The DS-ELISA required an optical scanner, a method of data analysis (usually computerised),

and accurate dilution equipment, preferably mechanical. The technical time required for performing each test was estimated at three minutes a sample for the DS-ELISA and two minutes a sample for the ISAGA, based on a batch run of 40 specimens.

Discussion

The dye test originated by Sabin and Feldman is the accepted reference assay used for the diagnosis of toxoplasmosis. This test measures both IgG and IgM antibodies, but a positive reaction does not delineate the immunoglobulin class. Although the dye test can be used as a reference value when assessing assays predominantly measuring toxoplasma specific IgG, it is of less value in the context of IgM detection.¹⁴ Samples showing a seroconversion from negativity to positivity are available in limited numbers only and the non-specific signs and symptoms of acute toxoplasmosis make diagnosis based on clinical findings unreliable.¹⁵ Analysis of antibody response using Western blots has failed to determine criteria for the diagnosis of acute toxoplasmosis¹⁶ while antigen detection and DNA probing techniques remain at the developmental stage. Isolation of the parasite from clinical samples does not differentiate acute from chronic infection. Consequently, there is no definitive reference to which toxoplasma IgM tests may be compared and calculation of predictive values or percentage figures for sensitivity and specificity is not justified.

Although both assay results were most often in agreement, the commonest finding in a confirmed mismatch was that of a positive ISAGA reaction in a sample negative by DS-ELISA testing. Our findings of persistence of ISAGA reactivity at higher serum dilutions and a greater period of time after acquisition of infection than by DS-ELISA suggest a proportion of discordant results will reflect the greater sensitivity of the former assay. Other groups have shown that ISAGA exhibits superior sensitivity compared with commercial ELISA systems¹⁷ or immunofluorescent antibody techniques.¹⁸ French workers found that 80% of patients maintained a positive ISAGA reaction one year after infection compared with 40% positive by commercial ELISA.¹⁷ In clinical practice toxoplasmosis is often considered only when more familiar disease processes have been excluded. Consequently, ISAGA would be useful for the investigation of suspected toxoplasmosis, particularly when the diagnosis is postulated late in the course of the infection.

Experience of ISAGA in Scotland has shown the persistence of reactivity into the second pregnancy following the birth of a congenitally infected infant.¹⁹ The risk of the parasite passing from mother to fetus

risks as the pregnancy progresses but the severity of any subsequent infection falls. Chronic latent toxoplasma infection of the mother poses no risk of congenital toxoplasmosis.²⁰ Although little data are available, it is thought that toxoplasmosis acquired before pregnancy will only result in congenital infection if parasitaemia persists at the time of conception. The duration of parasitaemia after the acquisition of infection is uncertain and is likely to vary both with the virulence of the parasite and the susceptibility of the host. Consequently the risk of fetal infection when conception occurs after the acquisition of toxoplasmosis, but at a time when specific IgM can be detected by a highly sensitive assay, such as ISAGA, has not been defined. Further studies are required so that suitable advice may be offered to pregnant women who produce a positive ISAGA reaction. Until such studies are completed, the investigation of these patients must include additional serological assessment.

The lack of a reference assay makes the interpretation of discordant ISAGA/DS-ELISA results problematic. We have shown that a proportion of samples producing a positive ISAGA reaction and a negative DS-ELISA result are likely to reflect the increased sensitivity of the former assay. Conversely, we have identified serial serum samples where the ISAGA reactivity has been lost before that of DS-ELISA. The two assays use different toxoplasma antigen profiles for the detection of specific IgM. Whereas ISAGA incorporates entire, formalin fixed trophozoites and mainly presents membrane antigens, DS-ELISA uses trophozoites disrupted by freeze-thawing, exposing both membrane and cytoplasmic antigens. Western blot analysis has shown a considerable variation in quantitative and qualitative antibody response to toxoplasma infection.²¹ Consequently some discordant IgM results will reflect the individual's immune response to selected antigens of the parasite and relative non-reactivity to other epitopes. It has been shown that haemolysed serum¹⁹ and excessive heat inactivation²² can result in false positive ELISA findings but the latter phenomenon has not been reported with μ chain capture assays. The problems associated with antinuclear and rheumatoid factor and IgM cross reactivity are well recognised, but we were unable to show erroneous ISAGA and DS-ELISA results in potentially difficult samples. Consequently we have no evidence to suggest discrepant DS-ELISA and ISAGA results represent false positive or false negative reactions by either assay. Previous studies have found the specificity of ISAGA to be superior to that of IFab test¹⁰ or Western blot analysis.²¹ Further studies of discordant toxoplasma specific IgM results are in hand.

The ease of performance, reproducibility, and sen-

sitivity of the ISAGA indicate that this test is suitable for the diagnosis of acute toxoplasma infection of immunocompetent subjects. In cases of suspected toxoplasmosis associated with pregnancy the ISAGA may be used as a screening test for recent infection but the persistence of ISAGA reactivity is such that further serological assessment will be required to define the risk of congenital infection. The value of ISAGA testing for the diagnosis of exacerbations of previously latent toxoplasmosis in immunocompromised patients requires evaluation.

We are indebted to Mrs I Crossman for her secretarial assistance.

References

- 1 Kean BH. Clinical toxoplasmosis—50 years. *Trans Roy Soc Trop Med Hyg* 1972;**66**:549–71.
- 2 Remington JS, Miller MJ, Brownlee I. IgM antibodies in acute toxoplasmosis II. Prevalence and significance in acquired cases. *J Clin Med* 1968;**71**:855–66.
- 3 Robertson PW, Kertesz V. Modified fluorescent antibody technique to detect immunoglobulin M antibodies to *Toxoplasma gondii* in congenital infection. *J Clin Microbiol* 1975;**2**:461–2.
- 4 Sato K, Ise Y, Iida T, Suzuki T, Shimada K, Nishioka K. Detection of toxoplasma IgM antibody by passive latex agglutination reaction. *J Immunol Methods* 1987;**101**:183–91.
- 5 Camargo ME, Ferreira AW, Mineo JR, Takiguti CK, Nakahara OS. Immunoglobulin G and immunoglobulin M enzyme-linked immunosorbent assays and defined toxoplasmosis serological patterns. *Infect Immun* 1978;**21**:55–8.
- 6 Naot Y, Remington JS. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. *J Infect Dis* 1980;**142**:757–66.
- 7 Naot Y, Barnett EV, Remington JS. Method for avoiding false-positive results occurring in immunoglobulin M enzyme-linked immunosorbent assays due to the presence of both rheumatoid factor and antinuclear antibodies. *J Clin Microbiol* 1981;**14**:73–8.
- 8 Desmonts G, Thulliez P. The toxoplasma agglutination antigen as a tool for routine screening and diagnosis of toxoplasma infection in the mother and infant. *Devel Biol Standard* 1985;**62**:31–5.
- 9 Pouletty P, Kadouche J, Garcia-Gonzalez M, et al. An anti-human μ chain monoclonal antibody: use for detection of IgM antibodies to *Toxoplasma gondii* by reverse immunosorbent assay. *J Immunol Methods* 1985;**76**:289–98.
- 10 Desmonts G, Naot Y, Remington JS. Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired toxoplasma infections. *J Clin Microbiol* 1981;**14**:486–91.
- 11 Payne RA, Isaac M, Francis JM. Enzyme-linked immunosorbent assay (ELISA) using antibody class capture for the detection of antitoxoplasma IgM. *J Clin Pathol* 1982;**35**:892–6.
- 12 Fleck DG, Kwantes W. *The laboratory diagnosis of toxoplasmosis*. Public Health Laboratory Service Monograph 13. London: HMSO, 1980:5–7.
- 13 Holliman RE, Johnson J, Duffy K, New L. Discrepant toxoplasma latex agglutination test results. *J Clin Pathol* 1989;**42**:200–3.
- 14 Johnson J, Duffy K, New L, Holliman RE, Chessum BS, Fleck DG. Direct agglutination test and other assays for measuring antibodies to *Toxoplasma gondii*. *J Clin Pathol* 1989;**42**:536–41.
- 15 Holliman RE, Fleck DG. Toxoplasma infection in the elderly. *Geriatr Med Today* 1986;**5**:97–102.

- 16 Partanen P, Turunen HJ, Paasivuo R, Ceinikki PO. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulins G, M and A antibodies at different stages of infection. *J Clin Microbiol* 1984;**20**:133-5.
- 17 Thulliez P, Dutriat P, Saulnier M, Vernes A, Desmonts G. Evaluation de trois réactifs de détection par immunocapture des IgM spécifiques de la toxoplasmose. *Rev Fr Lab* 1988;**169**: 25-31.
- 18 Desmonts G. Les recherches d'anticorps IgM spécifiques par immunoadsorption (immunosorbent assay au I.S.A.). *Lyon Med* 1982;**248**:37-41.
- 19 Skinner LJ, Chatterton JM, Joss AW, Moir IL, Ho-Yen, DO. The use of an IgM immunosorbent agglutination assay to diagnose congenital toxoplasmosis. *J Med Microbiol* 1989;**28**:125-8.
- 20 Desmonts G, Couvreur J. Toxoplasmose congenitale. Étude prospective de l'issue de la grossesse chez 542 femmes atteintes de toxoplasmose acquise en cours de gestation. *Ann Pediatr (Paris)* 1984;**31**:805-9.
- 21 Potasman I, Araujo FG, Thulliez P, Desmonts G, Remington JS. *Toxoplasma gondii* antigens recognized by sequential samples of serum obtained from congenitally infected infants. *J Clin Microbiol* 1987;**25**:1926-31.
- 22 Francis JM, Payne RA, Joynson DH, Balfour AH. False positive results with heated sera in toxoplasma ELISA for IgG antibody. *J Clin Pathol* 1987;**40**:356.

Requests for reprints to: Dr R E Holliman, Public Health Laboratory Service, Toxoplasma Reference Laboratory, St George's Hospital, London SW17 0QT, England.