Measurement of toxoplasma IgM by a microparticle capture enzyme immuno-assay

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Summary. The microparticle capture enzyme immuno-assay (MEIA) is an automated system for measuring specific antibody by interaction with antigen-coated particles. An MEIA method for detecting toxoplasma-specific IgM was compared with established reference methods. The MEIA had an acceptable level of sensitivity and reproducibility and was easier to perform than conventional tests, but it required expensive, dedicated equipment and, in our study, false positive results were recorded with 7% of samples. MEIA could be used to investigate immunocompetent patients with suspected toxoplasmosis but positive findings should be confirmed by an alternative form of assay. The technique is not suitable for the investigation of neonates, for which a more sensitive method is required.

Introduction

The major sequelae of Toxoplasma gondii infection in association with the acquired immunodeficiency syndrome (AIDS), organ transplantation and pregnancy have led to a demand for the serological assessment of large numbers of patients. Many of the current toxoplasma-specific antibody test methods involve macro-particle agglutination and are not amenable to automation. Enzyme-linked immuno-sorbent assays (ELISA) are suitable for processing large numbers of samples but require multiple manipulations. Consequently there is a need for new, precise, automated methods for measuring toxoplasma-specific immunoglobulin.

The microparticle capture enzyme immuno-assay (MEIA) was developed in an attempt to meet these criteria. The method involves the reaction of antigen-coated microparticles with the test serum, passage through a glass fibre matrix and detection of any antigen-antibody complex with enzyme-labelled anti-human immunoglobulin and a fluorescent substrate. The method is rapid and fully automated and the incorporation of anti-γ chain or anti-μ chain antibody permits the measurement of toxoplasma-specific IgG and IgM. The assessment of toxoplasma-specific IgG assays can be done by direct comparison with the dye test which is the accepted reference assay, or indirectly by comparison with a second method which has itself been validated against the dye test. Investigations of the measurement of toxoplasma-specific IgG by MEIA have been published. However, the evaluation of new methods for assay of toxoplasma-specific IgM is more complex because of the absence of a suitable reference assay. Assessment should preferably be by comparison with dye test findings in multiple series of samples that demonstrate seroconversion. Because of the limited availability of such specimens, assessment of a new assay must involve comparison with established methods and accumulation of "in use" experience. In this study, MEIA was compared with other established methods for the detection of toxoplasma-specific IgM.

Materials and methods

The microparticle capture enzyme immunoassay (MEIA)

The assay was fully automated (Abbott Laboratories Diagnostic Division) and was done in the sequence described below. The correct number of reaction cells required for the number of tests was loaded into a carousel by hand and 150 μl of each serum to be tested, plus two controls and a calibrator serum, were added by hand to separate sample wells. The automated process was then started. A 50-μl sample of the serum together with a diluent buffer were mixed in the dilution well. T. gondii-coated microparticles were added to the diluted serum and incubated to allow the microparticles to bind to the IgM, forming an antigen-antibody complex. This complex was transferred to a glass fibre matrix where the microparticles were irreversibly bound and the matrix was then washed to remove any unbound material. The automated system then added anti-human IgM

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conjugated with alkaline phosphatase, which bound to the antigen–antibody complex. The matrix was washed for a second time and 4-methyl-umbelliferyl phosphate was added, which reacted with the alkaline phosphatase to form a fluorescent product. The resulting fluorescence was recorded, the reading being directly proportional to the quantity of human IgM in the sample. The fluorescent signal was compared with a stored standard curve and the results were printed as the corresponding concentration, expressed as an index. All sera with indices of < 0·5 were regarded as negative.

Rheumatoid factor removed. All sera with indices > 0·5 were treated with the MEIA rheumatoid factor neutralisation reagent (IRFN), according to the manufacturer’s instructions; 50 μl of serum and 200 μl of IRFN were mixed and incubated at room temperature for 5–10 min and then centrifuged at 13000 g for 5 min. One hundred and fifty μl of each supernate was then re-assayed in a dilution assay.

Other tests

Toxoplasma-specific IgM was measured by a double sandwich enzyme-linked immunosorbent assay (DS-ELISA)\(^7\) and an immunosorbent agglutination assay (ISAGA)\(^8\). DS-ELISA results were expressed as enzyme immunoassay units (EIU) (< 25 = negative; 25–35 = equivocal; > 35 = positive) and ISAGA findings were recorded as an index score (0–5 = negative; 6–8 = equivocal; 9–12 = positive). The dye test (DT), latex agglutination test (LAT) and direct agglutination test (DAT) were performed as described previously.\(^8\)

Source of sera

Examination for acute toxoplasma-specific IgM was undertaken on routine specimens of serum submitted for the dye test and giving a result of > 31 IU (35·3%); on sera from HIV antibody-positive patients (22·9%); on transplant recipients (11·4%) and on the maternal sera of mother and baby pairs of cases of suspected congenital toxoplasmosis (16·3%). Samples from patients from suspected toxoplasma disease or unexplained lymphadenopathy (4·1%) and samples with DAT and LAT results which were suggestive of an acute infection were also examined. All sera were heated at 57°C for 30 min before assay to inactivate non-specific heat-labile factors.

Assessment of sensitivity and specificity

The sensitivity of the MEIA method was assessed further by taking five sera with a range of DS-ELISA values of 150–300 EIU and diluting these samples sequentially in DT-negative serum to a final concentration of 5% of the initial content. Each dilution was tested by MEIA, ISAGA and DS-ELISA. Potential false positive reactions were assessed by testing sets of five sera which gave negative results in toxoplasma serology (DT, LAT, DAT, DS-ELISA and ISAGA) but positive results for one of the following: anti-nuclear factor, rheumatoid factor, cytomegalovirus (CMV)-specific IgM, hepatitis A virus (HAV)-specific IgM. Five jaundiced and five haemolysed sera and 150 DT- and LAT-negative sera were excessively heat inactivated at 50°C for 3 h and then re-tested by MEIA, as were a further 150 DT- and LT-negative sera.

Five sera which gave positive results in all IgM tests, i.e., DS-ELISA, ISAGA, MEIA, were frozen and thawed five times and then re-tested to detect false negative reactions with MEIA.

Reproducibility

The reproducibility of the three IgM assays was assessed on 10 sera with DS-ELISA results of 0–300 EIU. Each sample was tested by all three assays on five separate occasions and the series was defined as reproducible if an identical result (positive, equivocal or negative) was recorded in all five tests by an individual assay.

Results

A total of 1011 routine samples was examined during the study; 646 (64%) gave negative results by MEIA, ISAGA and DS-ELISA and 80 (8%) gave positive results by all three methods. The majority of the positive results showed, as expected, that DS-ELISA was less sensitive than ISAGA. MEIA findings for this group of samples are presented in table I. The remaining 12 sera produced combinations of ISAGA and DS-ELISA results that did not reflect the comparative sensitivity of the two assays (table II).

Of five sera tested in dilutions from 100% to 5%, the ISAGA result remained positive at all dilutions, whereas the ELISA and MEIA findings became equivocal or negative at or below a 20% dilution.

None of the samples positive for CMV- or HAV-specific IgM, rheumatoid factor, anti-nuclear factor, nor the jaundiced nor the haemolysed sera gave a positive result when tested by the MEIA. However, seven of the additional 150 DT- and LAT-negative sera gave positive results and four equivocal results with the MEIA. DS-ELISA and ISAGA tests on these sera gave negative results. The five sera that were frozen and thawed five times remained positive when tested by all three assays for toxoplasma-specific IgM.

The 10 sera with DS-ELISA results of 0–300 EIU gave reproducible results with ISAGA on five separate occasions, and the findings for DS-ELISA and MEIA were consistent in eight and seven instances respectively.
Table 1. Comparison of MEIA, ISAGA and DS-ELISA findings in the measurement of toxoplasma-specific IgM.

<table>
<thead>
<tr>
<th>MEIA result</th>
<th>Number (%) of sera that were</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- E-</td>
<td>1- E+  I+ E-  1+ E+  I- E-  I- E- Total</td>
</tr>
<tr>
<td>+</td>
<td>80 (72) 5 (20) 10 (12) 11 (10) 19 (3) 125</td>
</tr>
<tr>
<td>±</td>
<td>17 2 12 15 6 52</td>
</tr>
<tr>
<td>-</td>
<td>14 18 61 83 646 822</td>
</tr>
<tr>
<td>Total</td>
<td>111 25 83 109 671 999</td>
</tr>
</tbody>
</table>

1. ISAGA: E, DS-ELISA: +, positive; ±, equivocal; -, negative.

Table 11. Analysis of discordant MEIA, ISAGA and DS-ELISA findings.

<table>
<thead>
<tr>
<th>MEIA result</th>
<th>Number (%) of sera that were</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- E-</td>
<td>1- E+  I+ E-  1+ E+  I- E-  I- E- Total</td>
</tr>
<tr>
<td>+</td>
<td>5 1 0 6</td>
</tr>
<tr>
<td>±</td>
<td>2 0 0 2</td>
</tr>
<tr>
<td>-</td>
<td>2 0 2 4</td>
</tr>
<tr>
<td>Total</td>
<td>9 1 2 12</td>
</tr>
</tbody>
</table>

1. ISAGA: E, DS-ELISA: +, positive; ±, equivocal; -, negative.

The analyser took c. 45 min to process a full carousel of 21 test sera. The absorption procedure was relatively labor-intensive and took a further 90 min to process 21 specimens. The MEIA methodology was less technically demanding than either DS-ELISA or ISAGA.

Discussion

There is no readily available, definitive reference method with which new toxoplasma IgM assays may be compared. Serial samples showing dye test seroconversion are rarely available and the clinical diagnosis of acute infection is unreliable; nor does isolation of the parasite separate acute from chronic infection. Therefore, it is not possible to calculate absolute values for the sensitivity, specificity and predictive value of the MEIA assay for toxoplasma-specific IgM.

The DS-ELISA employed in the current study is used routinely at three toxoplasma reference laboratories in England and Wales: the ISAGA is an established French reference assay. It has been shown that the ISAGA is significantly more sensitive than DS-ELISA: reactivity persists for 18 months by the former assay, compared to an average of 6–8 months when tested by the DS-ELISA in our laboratory. The ISAGA incorporates entire, intact toxoplasma trophozoites as an antigen source and the DS-ELISA antigen consists of disrupted organisms. Consequently the antibody profile detected by each assay is not identical and occasionally discordant findings are noted. The current study has shown that the sensitivity of the MEIA is comparable to that of DS-ELISA but is significantly less sensitive than ISAGA. We would expect MEIA reactivity to persist for c. 8 months after acute exposure to the parasite, but this aspect requires confirmation by serial testing of sera from individuals with acute infection.

The MEIA produced a positive or equivocal reaction in 7% of samples which were non-reactive in all other assays of toxoplasma-specific antibody, including the dye test. Although detectable IgM production may precede that of toxoplasma-specific IgG, this immune response should have been detected by a highly sensitive assay such as ISAGA. Furthermore, the dye test would have detected complement fixing IgM. Serial samples were not available from these patients and so we could not assess the progress of this immune response as detected by MEIA. However, it is most likely that these MEIA reactions represented false positive results. We were unable to associate discordant findings with non-toxoplasma-specific IgM, physical appearance of the sample or laboratory methods for inactivating or storing sera. Although MEIA gave less reproducible findings than the other two methods, this may reflect our lack of experience with a newly introduced technique.

Performance of the MEIA was less demanding than ISAGA or DS-ELISA. This reduction in labour costs is offset by the additional expense of reagents and a dedicated analyser.

Another "in-house" assessment of MEIA has been published which processed fewer samples than the present investigation and compared MEIA findings with a commercial IgM ELISA. Discordant results were resolved by using ISAGA. Ten cases of IgG and IgM seroconversion were included in the study but these were the only samples assessed by the dye test. The MEIA gave a high degree of concordance with the commercial IgM ELISA, but this IgM ELISA has not been subjected to a detailed comparison with other well established assays. Notably, the incidence of IgM MEIA reactivity in samples lacking antibody detectable by other assays was not investigated. Because of these factors it is difficult to compare the conclusions of the two studies.

Our results indicate that the MEIA method is suitable for the investigation of acute toxoplasma infection of immunocompetent patients, recipients of organ grafts and pregnant women. Reactive samples should be assessed by a second assay to ensure specificity. In congenital infection the neonatal IgM response is best detected by an assay of maximal sensitivity such as ISAGA.

The ease of operation and use of the analyser for multiple assays indicate that MEIA will be of value to the routine laboratory. The reference centre dealing with specialised toxoplasma investigation will find the MEIA automated format and level of sensitivity suitable for processing large numbers of samples.
MEIA toxoplasma reagents and automated analyser were provided by Abbott Laboratories. We are grateful to Mrs D. Lyndon for her secretarial assistance and to D. A. Hamilton for computer analysis of the results.

References