How to detect current toxoplasma infection

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Accepted: 19 October 2001

ABSTRACT

This study seeks to identify the best way to detect current toxoplasma infection for district general hospital laboratories. One hundred 'ordinary' and 174 'difficult' sera are categorised into either an 'evidence' or 'no evidence' group for current toxoplasma infection. Twelve test protocols are investigated using different combinations of one whole antibody latex test (Eiken Toxoreagent), one in-house specific IgG enzyme-linked immunosorbent assay (ELISA) and three specific IgM assays (Toxo-ISAGA, in-house BAM ELISA IgM and Toxonostika ELISA M). The Eiken latex and in-house IgG assays produced significantly fewer false-negative results than were obtained with the single IgM test or the IgG and IgM test protocols ($P<0.05$), but a greater number of false-positive results (102/274 and 115/274, respectively). Of the IgM assay test protocols, the three IgM assays in combination produced the least number of false-negative results (1/274). However, a significantly greater number of false-positive results were produced than with one or two IgM tests or an IgG and any IgM test in combination ($P<0.001$). We recommend testing with three IgM tests, or a whole antibody (Eiken) or IgG-specific assay, and that positive or clinically important negative samples be referred to a reference laboratory for confirmation.

KEY WORDS: Diagnosis. IgM. Laboratory techniques and procedures. Referral and consultation. Toxoplasma.

Introduction

A changing pattern of testing for toxoplasma infection has been seen in district general hospital (DGH) laboratories over the last 10 years in the UK.[1] In the past, many DGH laboratories would either refer all samples to a reference laboratory or screen them using a specific IgG assay or whole antibody assay for toxoplasma, referring only positive samples to a reference laboratory for further testing. More recently, however, there has been an increasing trend for DGH laboratories also to test for toxoplasma-specific IgM.

A total of 14 assays are used by 47 users of the toxoplasma IgM UK National External Quality Assurance Scheme (UK NEQAS), but there is a skewed distribution of assay manufacturer: Abbott AxSym Toxo M ($n=14$), bioMérieux VIDAS IgM ($n=13$), bioMérieux Toxo-ISAGA ($n=5$), two have two users and nine have a single user (Chiodini PL, Kettlehut M: personal communication). As IgM assay performance is variable,[2-7] the diversity of assays in use in the UK raises concern about the diagnostic accuracy to current toxoplasma infection.

Sensitivity of the IgM assay appears to be a problem. Too sensitive an assay may wrongly suggest infection during pregnancy rather than before conception, when only infection during pregnancy has serious, even fatal, consequences.[8] However, a very sensitive assay is necessary for the detection of toxoplasma-specific IgM among neonates and immunocompromised patients.[9]

Users of the Scottish Toxoplasma Reference Laboratory (STRL) have asked for guidance about the best way to detect current toxoplasma infection in a DGH laboratory, and this study examines the options available.

Materials and methods

Sera

Sera received at STRL between 1 January 2000 and 31 December 2000 were studied, representing those that a DGH laboratory might expect to receive. One hundred 'ordinary' sera were selected randomly from 100 patients seen by local general practitioners or by clinicians in local hospitals. A second group included 174 'difficult' sera from 138 patients and initially tested in other laboratories. The 'ordinary' sera group was characterised as toxoplasma specific antibody-positive or -negative using a modification of the Sabin-Feldman dye test.[10] Selection criteria used for the 'difficult' sera were either a positive result in the STRL screening BAM ELISA IgM (BAM) or a dye test >125 IU/mL (normal range 0-125 IU/mL) regardless of the BAM result.

Evidence of current toxoplasma infection in the two groups was identified in a total of 72 sera ('ordinary' group, zero; 'difficult' group, $n=72$). These were categorised using a range of tests available at STRL (dye test, ELISA IgG, BAM ELISA IgM, immunosorbent agglutination assay [ISAGA] IgM, IgA, IgE, Toxonostika ELISA IgM [EM], IgG avidity) and the submitted clinical information. The criteria for evidence of current infection, as agreed with the Scottish Centre for Infection and Environmental Health, are: a positive dye test and positive EM, or a positive ISAGA IgM in neonates or in the immunocompromised.

Testing protocols

All sera were tested by a whole antibody latex test (Eiken Toxoreagent, Eiken Chemical Co., Japan), an in-house specific IgG ELISA,[11] a very sensitive IgM assay (Toxo-ISAGA IgM, bioMerieux, France), a moderately sensitive IgM assay (in-house BAM ELISA IgM)[12] and a low-sensitivity IgM assay

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(Toxonostika ELISA IgM, Organon Teknika, UK). The assays were performed as previously described or according to the manufacturer's instructions. In all cases, equivocal results were considered as positive as the patient management would be similar.

Twelve test protocols were used: each test individually (n=5), a combination of the IgG assay and one IgM assay (n=3), or a combination of two IgM assays (n=3) or three IgM assays (n=1). For each test protocol using one or two assays, a result was regarded as positive when all tests were positive; and for the three IgM assay test protocol, two assays had to be positive. In addition, for the IgG and IgM combinations, a negative IgG test and positive IgM test was regarded as a positive result.

Statistics

Results were analysed using the Chi-squared (χ^2) test.[13]

Results

The sensitivity and specificity of individual assays are presented in Table 1. The false-positive, true-positive and false-negative results of the different test protocols are presented in Table 2. Two samples from the category with evidence of current infection were of insufficient volume to test using the Eiken latex assay.

There were significantly fewer false-negative results with the Eiken and IgG tests, compared with an IgM test alone, with IgG and IgM tests in combination (Table 2, test protocols 3-8, $P<0.05$), or with two IgM tests (Table 2, test protocols 9-11, $P<0.05$). However, the use of three IgM tests produced only one false-negative result (this patient had a raised dye test result of 250 IU/mL and one positive IgM test).

The Eiken, ELISA IgG and three IgM test combination protocol produced a significantly higher number of false-positive results, compared with one IgM test alone, one IgG and one IgM test in combination (Table 2, test protocols 3-8, $P<0.05$) or two IgM tests in combination (Table 2, test protocols 9-11, $P<0.001$). No differences were observed using one IgG and one IgM test in combination, compared with results obtained with a single IgM test (Table 2).

Two IgM tests reduced the number of false-positive results, particularly if the most sensitive and least sensitive assays were used in combination (Table 2, test protocol 10). However, the number of false negatives was greater than that obtained using an IgM test alone or an IgG test and any one IgM test in combination.

Table 1. Sensitivity and specificity of one total antibody, one IgG and three IgM tests relative to dye test results on 100 'ordinary' sera

Test	Number of sera positive by test/dye test-positive sera (sensitivity, %)	Number of sera negative by test/dye test-negative sera (specificity, %)
Eiken	19/22 (86)	76/78 (97)
EG	21/22 (95)	71/78 (91)
ISAGA	6/22 (27)	76/78 (97)
BAM	3/22 (14)	75/78 (96)
EM	0/22 (0)	75/78 (96)

Table 2. Evidence of infection in 274 screened samples by the 12 test protocols

Test protocol	False positive with test protocol	True positive with test protocol and referral	False negative with test protocol
Total antibody test			
1. Eiken	102	70*	0
IgG test only			
2. EG	115	72	0
IgM test only			
3. ISAGA	40	66	6
4. BAM	81	65	7
5. EM	7	68	4
IgG and IgM tests			
6. EG + ISAGA	40	66	6
7. EG + BAM	81	65	7
8. EG + EM	7	68	4
Two IgM tests			
9. ISAGA + BAM	23	59	13
10. ISAGA + EM	2	63	9
11. BAM + EM	9	62	10
Three IgM tests			
12. ISAGA +BAM+EM	96	71	1

** insufficient sera in two samples for Eiken test*

Discussion

Seroprevalence of 22% for toxoplasma is slightly higher than previously reported for the Highlands of Scotland (17%),[14] and this may have be due to the relatively small sample size (n=100) or have been influenced by the age distribution of the sample population.

Sensitivity and specificity values of the in-house ELISA IgG and the Eiken latex tests were similar to previously reported results.[15] The dye test values of the three false-negative Eiken results and the single IgG ELISA result were at or below the threshold of each assay (15 IU/mL and 8 IU/mL, respectively; Table 1). The IgM tests exhibited a gradation of sensitivity, confirming the variability between these tests.[2-7] The ISAGA was most sensitive, the BAM moderately sensitive and the EM the least sensitive test, the BAM sensitivity being similar to other commercial assays.[2]

Positive results from the 100 'ordinary' sera were all in patients not in the 'current infection' category, demonstrating that ISAGA and some ELISA assays for IgM (e.g. BAM) can continue to produce positive results many months after onset of infection.

The use of one IgM assay in the diagnosis of current toxoplasma infection can have serious consequences for patient management.[2,16] A retrospective cohort study of 811

consecutive pregnant women was performed at a toxoplasma reference laboratory in the USA,[16] and the results indicated that the rate of unnecessary induced abortions could be reduced by approximately 50% in women who were toxoplasma specific IgM-positive at referring laboratories if confirmatory serological IgM testing was performed at a reference laboratory. The group concluded that the practice of referring positive samples to a reference laboratory for confirmatory diagnostic testing would improve the management of toxoplasmosis in pregnant women.

From our data, it is apparent that the use of any of the different test protocols alone would result in an inaccurate diagnosis, and a total antibody or IgG-specific test, or a combination of three IgM tests, gives rise to excessive false-positive results. Although the test protocols that include one or two IgM tests, or a combination of an ELISA IgG and one IgM test, reduce the number of false-positive results, a significant number of patients with current infection would not be diagnosed.

The best protocol is to test with a total antibody or specific IgG test and then refer positive samples to a reference laboratory for confirmation. There is no advantage in testing for IgM and then referring the sample on as false-negative results are produced. Although the use of three IgM tests reduces the number of false-negative results, the higher cost implications and high false-positive rate would need to be considered.

The use of a single test such as the Eiken or ELISA IgG can produce false-negative results in patients with very acute onset of symptoms. It is recognised that IgM can be detected before the IgG response[9] and therefore is recommended for patients with clinical toxoplasma in whom the IgG or Eiken test is negative, following which the sample should be referred to the reference laboratory for further testing. Although the Eiken latex assay can detect total toxoplasma-specific antibody, this advantage may be limited as the threshold value is relatively high compared with some IgG assays.

The role of the reference laboratory does not compete with that of the DGH laboratory but is supportive of the service it provides to users.[17] However, the provision of extensive methods of testing is only part of this support, which also includes expert clinical advice, staff who have experience with unusual and problematic clinical and technical situations, and ongoing evidence-based research programmes aimed at improving diagnosis.[15]

In conclusion, both the reference laboratory and DGH laboratory need to develop appropriate testing and referral protocols to improve patient management of this serious infection. □

We are indebted to all our users for referring samples to the reference laboratory and to the National Services Division at the Scottish Office for their financial support.

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