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Study of Abbott Toxo IMx System for Detection of Immunoglobulin G and Immunoglobulin M Toxoplasma Antibodies: Value of Confirmatory Testing for Diagnosis of Acute Toxoplasmosis

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We compared the Abbott Toxo immunoglobulin G (IgG) and IgM IMx assays with the Sabin-Feldman dye test and an IgM enzyme-linked immunosorbent assay (ELISA) in 398 serum samples previously tested in our laboratory (retrospective group) and 1,000 consecutive serum samples, tested as they were received in our laboratory in 1995 (prospective group). In the retrospective group, the IgG IMx had a sensitivity of 100%, specificity of 99.0%, positive predictive value of 99.0%, negative predictive value of 100%, and overall agreement of 99.5%. The percentages for the IgM IMx were 97.8, 99.0, 98.9, 98, and 98.4%, respectively. In the prospective group, the IgG IMx had a sensitivity of 97.8%, specificity of 98.7%, positive predictive value of 97.8%, negative predictive value of 98.7%, and overall agreement of 98.4%. The percentages for the IgM IMx were 88.3, 95.9, 74.7, 98.3, and 95%. A serological profile (IgA and IgE antibodies and the differential agglutination [AC/HS] test) was performed in an attempt to resolve discrepancies. Of 21 serum samples that were positive in the IgM IMx and negative in the IgM ELISA, 19 had serological profiles which were most compatible with an infection acquired in the distant past. Of 8 serum samples which were positive in the IgM ELISA and negative in the IgM IMx, 5 had serological profiles which were most compatible with an infection acquired in the distant past. Of the 55 serum samples that were positive in both IgM tests, 21 were from patients who had serological profiles which were most compatible with an infection acquired in the distant past. In conclusion, our data highlight the importance of confirmatory testing for the diagnosis of recently acquired infection with *Toxoplasma gondii*. When compared with the dye test and IgM ELISA, the Toxo IgG and IgM IMx assays, respectively, revealed high overall agreement in the retrospective and prospective study.

Serologic tests for detection of immunoglobulin G (IgG) and IgM antibodies are commonly performed for the diagnosis of acute acquired toxoplasmosis in the immunocompetent patient (13). A variety of methods which vary considerably in their sensitivity and specificity are being used for this purpose (11, 13). A positive IgG titer in most cases is sufficient to establish that a patient has been infected with *Toxoplasma gondii*. Because IgM antibodies may persist for ≥1 year following the acute infection (2, 5), their greatest value is that a negative result virtually rules out a recently acquired infection, unless sera are tested so early that an antibody response has not yet developed or is undetectable.

Commercial kits for detection of toxoplasma antibodies are increasingly being used. A potential problem with many of these kits is the exceedingly high number of false positive and false negative results (1, 17). Since interpretation of serologic test results for the diagnosis of toxoplasmosis in pregnant women in the United States and many other nations is most often based on results obtained in a single serum sample, misinterpretation of a positive result, especially in an IgM antibody test, can and has led to unnecessary concern and abortion (3, 12).

One of the most widely used commercial methods worldwide is the Abbott IMx. To evaluate the improved version of the Abbott Toxo IgG and IgM IMx tests (version 2.0), we compared them with the Sabin-Feldman dye test (14) and an IgM enzyme-linked immunosorbent assay (ELISA) (originally developed in our laboratory [10]), respectively. Comparisons were performed both retrospectively on selected sera that had previously been tested in our laboratory and prospectively on consecutive sera from adults that had been received by our laboratory for diagnostic testing. In order to investigate the significance of detection of IgM antibodies by either method, results obtained in additional serological tests, including IgA (15) and IgE ELISAs (18), IgE immunosorbent agglutination assay (ISAGA) (18), and differential agglutination (AC/HS) tests (4), as well as results of follow-up serological tests, were analyzed.

MATERIALS AND METHODS

Sera. Sera were submitted from outside sources to our Toxoplasma Serology Laboratory at the Palo Alto Medical Foundation (PAMF). Diagnoses in these patients included pregnancy (36%), lymphadenopathy (2.1%), AIDS (1.8%), and a variety of other conditions (5.3%). There was no clinical information provided with 55% of the samples. Sera were divided into two groups as follows.

The retrospective group consisted of sera previously tested in our laboratory. These had been stored at −20°C for up to 5 years; most (>80%) had been stored for less than 3 years. In this group, 203 serum samples were positive for IgG antibodies, 195 were negative for IgG antibodies, 187 were positive for IgM antibodies, and 206 were negative for IgM antibodies. The 187 serum samples which were positive for IgM antibodies were also positive for IgG antibodies.

The prospective group comprised 1,344 consecutive serum samples from adults. These sera were received in our laboratory for diagnostic testing over a

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TABLE 1. Results for the retrospective group

Comparison assay	IgG IMx test result (%)[a]			IgM IMx test result (%)[b]		
	positive	equivocal	negative	positive	equivocal	negative
Sabin-Feldman dye test						
positive	203 (51.0%)	0	0			
negative	2 (0.5%)	2 (0.5%)	191 (48.0%)			
IgM ELISA						
positive				174 (44.3%)	9 (2.3%)	4 (1.0%)
equivocal				1 (0.3%)	2 (0.5%)	0
negative				2 (0.5%)	4 (1.0%)	197 (50.1%)

[a] $n = 398$.
[b] $n = 393$.

period of 4 months in 1995. Of these, 330 (24.6%) were excluded because of an insufficient quantity of serum. An additional 14 (1.0%) with hemolysis or lipemia were excluded from testing according to the protocols included with each IMx kit. Sera were stored at 4°C and tested within 5 days of receipt.

In addition, we examined 29 serial serum specimens from seven women who had seroconverted during gestation.

Abbott IMx. The Abbott IMx uses an automated system which is based on microparticle enzyme immunoassay technology to detect antibodies to *T. gondii*. The improved versions of the IgG and IgM IMx tests (IMx Toxo IgM and IgG, version 2.0) were performed as directed by the manufacturer. After manual pipetting of sera into individual reaction cells, all subsequent steps are automated. The instrument delivers the sample and diluent buffers to the predilution well of the reaction cell. *T. gondii*-coated microparticles and diluted sample are added to the incubation well, where the *T. gondii* antibody binds to the *T. gondii*-coated microparticles, forming an antigen-antibody complex. A diluted aliquot of this antigen-antibody complex is transferred to the glass fiber matrix, which irreversibly binds the microparticles. After washing the matrix to remove unbound materials, class-specific anti-human IgG-alkaline phosphatase conjugate is dispensed onto the matrix, where it binds to the antigen-antibody complex. The matrix is again washed to remove unbound materials; this is followed by addition of the substrate, 4-methylumbelliferyl phosphate. The resulting fluorescent product is measured by the microparticle enzyme immunoassay optical assembly. The intensity of the fluorescence is proportional to the quantity of toxoplasma antibodies. Those specimens with positive results in the IgM assay were treated with the rheumatoid factor neutralization reagent according to the manufacturer's instructions in order to remove possible rheumatoid factor activity.

Results are expressed as international units per milliliter (IU/ml) in the IgG assay and as an index in the IgM assays. This index is derived by dividing the patient specimen value by the value of the index calibrator included in each run. Interpretation of IgG IMx results was based on the manufacturer's criteria as follows: >3.0 IU/ml, positive for IgG antibodies; 2.0 to 3.0 IU/ml, equivocal result; and <2.0 IU/ml, negative for IgG antibodies. IgM IMx results of <0.500 were considered negative. Those sera with results of ≥0.500 were neutralized with the rheumatoid factor neutralization reagent and interpreted by using the manufacturer's criteria as follows: ≥0.600, positive; 0.500 to 0.599, equivocal; and <0.500, negative.

IMx precision was evaluated by intra-assay and interassay testing of negative and positive controls in each assay. Intra-assay precision was determined by running three consecutive runs of each negative and positive control for IgG and IgM antibodies. Interassay precision was determined by testing one negative and one positive control, each in duplicate, for 10 days. IMx reproducibility was determined by testing a panel of three serum samples coded in a blinded manner, each in triplicate, on 3 consecutive days.

PAMF serological tests. The Sabin-Feldman dye test (14), direct agglutination test (6), double-sandwich IgM ELISA (10), IgA ELISA (15), IgE ISAGA (18), IgE ELISA (18), and AC/HS (4) were performed as previously described. The Sabin-Feldman dye test is considered the "gold standard" for detection of IgG antibodies against *T. gondii*. While there is no comparable reference method for detection of IgM antibodies, the double-sandwich IgM ELISA method developed by our laboratory has been shown to be more sensitive and specific than conventional IgM ELISAs and the IgM immunofluorescence assay, and it agrees well with a battery of confirmatory methods (9, 19). It has been used by our laboratory and other reference laboratories for more than 15 years. In the dye test, a titer of <1:16 was considered negative and a titer of ≥1:16 was considered positive. In the IgM ELISA, a result of ≥2.0 was interpreted as positive, a result of 1.7 to 1.9 was interpreted as equivocal, and a result of <1.7 was interpreted as negative. Results in the IgA ELISA of ≥2.1 were considered positive, results of 1.5 to 2.0 were considered equivocal, and results of ≤1.4 were considered negative. The AC/HS test was interpreted as previously described (4) by comparing titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen). IgG antibodies formed early in infection recognize stage-specific antigens in the AC preparation which are distinct from those formed later in infection (4, 9, 16).

Testing protocol. Version 2.0 IMx test kits were provided by Abbott Laboratories (Abbott Park, Illinois) and were used according to the manufacturer's protocol.

For sera in the retrospective group, when discrepancies were found between results obtained in the IgG or IgM IMx and the respective PAMF test, the dye test or IgM ELISA was repeated. This was to confirm that the results obtained in the PAMF test had not changed in the interval between original testing and testing in the IMx. The repeat test results were used in this study. When discrepancies were observed between repeat test results and IgM IMx results, an IgA ELISA and an AC/HS test were performed to aid in their resolution.

For sera in the prospective group, we attempted to resolve discrepancies between results obtained in the IgM IMx and IgM ELISA by using results obtained in the PAMF serological profile (IgA and IgE ELISA, IgE ISAGA, and AC/HS). Two investigators interpreted in a blinded manner results obtained in the PAMF serological profile. Test results were interpreted as being one of the following: (i) patients with a serological profile which was most compatible with an infection acquired recently, (ii) patients with a serological profile which was most compatible with an infection acquired in the distant past, or (iii) patients whose serological profile was considered equivocal. When available, results obtained in the testing of follow-up sera were also used. Sera for which IgA and AC/HS test results were not available were excluded from this analysis. In our laboratory, it is routine practice to test sera from adults for both IgG and IgM antibodies. When these results indicate the possibility of a recent infection, the referring physician is contacted to request approval for the PAMF serological profile, including IgA, AC/HS, and IgE tests, to assist us in evaluating whether the patient may have recently been infected. In some cases, additional testing was not approved by the corresponding physician, whereas in others, the PAMF serological profile was ordered initially.

Since no reference method for testing of IgM antibodies has been established, we were also interested in evaluating how all of the IgM results related to those in the PAMF serological profile. For this purpose, for those sera positive in both IgM methods, we also compared IgM ELISA and IgM IMx results with those obtained in the PAMF serological profile.

Technical performance comparison. Sensitivity was defined as the percentage of specimens positive in the PAMF assay that were identified as positive by the IMx test. Specificity was defined as the percentage of specimens negative in the PAMF assay that were identified as negative in the IMx test. The positive predictive value was defined as the probability that a positive IMx result would be positive in the PAMF assay. The negative predictive value was defined as the probability that a negative IMx result would be negative in the PAMF assay. Overall agreement was defined as the percentage of specimens that were positive or negative in the PAMF assay and gave the same positivity and negativity in the IMx assay (7).

Statistics. The coefficient of variation was used to determine intra- and interassay precision and reproducibility. The coefficient of correlation (r) was used to determine the statistical agreement between the different tests.

RESULTS

Retrospective study. Results of the comparison of the IgG IMx and IgM IMx with the dye test and IgM ELISA, respectively, are shown in Table 1.

(i) IgG antibodies. Of the 398 serum samples, 203 (51.0%) were positive in the dye test. Each of the latter serum samples was positive in the IgG IMx. Of the 195 (49.0%) serum samples that were negative in the dye test, 191 (97.9%) were negative and 2 (1%) were positive in the IgG IMx. Results in the IgG

TABLE 2. Discrepant results between the IgM IMx and IgM ELISA in the retrospective group

Serum sample	Dye test titer[a]	Value (result) for:			
		IgM IMx[b]	IgM ELISA[c]	IgA ELISA[d]	AC/HS[e]
71	32	0.755 (+)	1.6 (−)	1.5 (E)[f]	50/400 (NA)
201	256	0.611 (+)	1.0 (−)	2.0 (E)	50/800 (NA)
410	128	0.495 (−)	2.5 (+)	2.1 (+)	<50/200 (NA)
421	512	0.321 (−)	2.8 (+)	1.8 (E)	100/200 (A)[g]
471	1,024	0.494 (−)	4.0 (+)	5.5 (+)	800/800 (A)
526	8,000	0.445 (−)	5.0 (+)	4.2 (+)	>1,600/>3,200 (A)

[a] Values are reciprocal of titers.
[b] Results of <0.500 were considered negative. Those sera with results of ≥0.500 were neutralized with rheumatoid factor neutralization reagent and interpreted according to the manufacturer's criteria as follows: ≥0.6, positive; 0.5 to 0.599, equivocal; and <0.5, negative.
[c] A result of ≥2.0 was interpreted as positive, a result of 1.7 to 1.9 was interpreted as equivocal, and a result of <1.7 was interpreted as negative.
[d] Results of ≥2.1 were considered positive, results of 1.5 to 2.0 were considered equivocal, and results of ≤1.4 were considered negative.
[e] NA, non-acute pattern; A, acute pattern.
[f] E, equivocal result.
[g] Borderline result.

IMx were equivocal for two serum samples (0.5%). Sera yielding equivocal results were excluded from the remainder of the analysis.

Discrepant results were noted for two serum samples that were negative in the dye test and positive in the IgG IMx. In an attempt to resolve these discrepancies, the dye test was repeated in twofold dilutions between undiluted and 1:8. In both serum samples, the dye test was positive at 1:8. These sera also gave positive results in the direct agglutination test. When compared with the dye test, the IgG IMx had a sensitivity of 100%, specificity of 99.0%, positive predictive value of 99.0%, negative predictive value of 100%, and overall agreement of 99.5% ($r = 0.99$).

(ii) IgM antibodies. Of the 393 serum samples tested, 187 (47.6%) were positive in the IgM ELISA. Of the latter sera, 174 (93.0%) were positive and 4 (2.1%) were negative in the IgM IMx. Of the 203 (51.6%) serum samples that were negative in the IgM ELISA, 197 (97.0%) were negative and 2 (1.0%) were positive in the IgM IMx. Results in the IgM ELISA were equivocal in three (0.8%) serum samples which had originally tested negative for IgM antibodies. Results in the IgM IMx were equivocal in 15 (3.8%) serum samples. Two serum samples gave equivocal results in both tests. Sera with equivocal results were not used in the remainder of the analysis.

The discrepant results, noted in 6 serum samples, are shown in Table 2. Each was positive in the dye test, 2 were positive in the IgM IMx and negative in the IgM ELISA, and 4 were negative in the IgM IMx and positive in the IgM ELISA. Two of the serum samples (samples 71 and 201) which were positive in the IgM IMx and negative in the IgM ELISA were considered most likely to have come from patients infected in the distant past, since the AC/HS test was negative and the IgA ELISA was equivocal. Three of the serum samples (samples 421, 471, and 526) that were negative in the IgM IMx and positive in the IgM ELISA were considered likely to have come from patients with relatively recent infections, since the AC/HS test was positive in all cases and the IgA ELISA was positive in two cases and equivocal in one case. Resolution of discrepancies in one case (sample 410) was not possible since the IgA ELISA and the AC/HS test did not give concordant results. When compared with the IgM ELISA, the IgM IMx had a sensitivity of 97.8%, a specificity of 99%, a positive predictive value of 98.9%, a negative predictive value of 98%, and an overall agreement of 98.4% ($r = 0.97$).

Prospective study. Results of the comparison of the IgG IMx and IgM IMx with the dye test and IgM ELISA, respectively, are shown in Table 3.

(i) IgG antibodies. Of the 1,000 serum samples tested, 356 (35.6%) were positive in the dye test, and of these, 348 (97.8%) were positive and 8 (2.2%) were negative in the IgG IMx. Of the 644 (64.4%) serum samples that were negative in the dye test, 630 (97.8%) were negative and 8 (1.2%) were positive in the IgG IMx. Results in the IgG IMx were equivocal in six (0.6%) serum samples. The latter sera were not used in the remainder of the analysis.

Discrepant results were noted in 16 serum samples. Eight were positive in the dye test and negative in the IgG IMx; 8 were negative in the dye test and positive in the IgG IMx. In an attempt to resolve these discrepancies, the dye test was repeated in twofold dilutions between undiluted and 1:8 for the sera with negative results in the dye test and positive results in the IgG IMx. In 7 of the 8 serum samples, the dye test was positive between undiluted and 1:8. Of these 7 serum samples, 6 could also be tested in the direct agglutination test (one serum sample could not be tested because of an insufficient quantity of serum). The direct agglutination test was positive for four serum samples and negative for two serum samples. Only one serum sample that tested positive in the IgG IMx gave repeatedly negative results when retested in the dye test in lower dilutions. When compared with the dye test, the IgG IMx had a sensitivity of 97.8%, a specificity of 98.7%, a positive predictive value of 97.8%, a negative predictive value of 98.7%, and an overall agreement of 98.4% ($r = 0.97$).

(ii) IgM antibodies. A total of 95 (13.9%) of the 684 serum samples tested were positive in the IgM ELISA. Of these, 68

TABLE 3. Results for the prospective group

Comparison assay	IgG IMx test result (%)[a]			IgM IMx test result (%)[b]		
	positive	equivocal	negative	positive	equivocal	negative
Sabin-Feldman dye test						
positive	348 (34.8%)	0	8 (0.8%)			
negative	8 (0.8%)	6 (0.6%)	630 (63%)			
IgM ELISA						
positive				68 (9.9%)	18 (2.6%)	9 (1.3%)
equivocal				8 (1.2%)	4 (0.6%)	3 (0.4%)
negative				23 (3.4%)	15 (2.2%)	536 (78.4%)

[a] $n = 1,000$.
[b] $n = 684$.

(71.6%) were positive and 9 (9.5%) were negative in the IgM IMx. Of the 574 (83.9%) serum samples that were negative in the IgM ELISA, 536 (93.4%) were negative and 23 (4.0%) were positive in the IgM IMx. Results in the IgM ELISA were equivocal for 15 (2.2%) serum samples; results in the IgM IMx were equivocal for 37 (5.4%) serum samples. Four serum samples gave equivocal results in both tests. Sera with equivocal results were not used in the remainder of the analysis.

Of the 32 serum samples with discrepant results, 9 were positive in the IgM ELISA and negative in the IgM IMx, and 23 were negative in the IgM ELISA and positive in the IgM IMx. When compared with the IgM ELISA, the IgM IMx had a sensitivity of 88.3%, specificity of 95.9%, positive predictive value of 74.7%, negative predictive value of 98.3%, and an overall agreement of 95% ($r = 0.84$). We attempted to resolve discrepancies that occurred between the IgM ELISA and the IgM IMx. For this purpose, we evaluated results obtained in the PAMF serological profile (IgA ELISA, AC/HS, IgE ELISA, and IgE ISAGA) to aid in assessing the likelihood of a recently acquired infection. Results obtained in the PAMF serological profile and/or results of follow-up specimens were available for 29 (90.6%) of the 32 discrepant serum samples. Of eight serum samples that were positive in the IgM ELISA and negative in the IgM IMx, two (25%) had PAMF serological profiles which were most compatible with an infection acquired recently, five (62.5%) had PAMF serological profiles which were most compatible with an infection acquired in the distant past, and in one (12.5%), results were considered equivocal. Of 21 serum samples that were positive in the IgM IMx and negative in the IgM ELISA, 1 (4.8%) had a PAMF serological profile which was most compatible with an infection acquired recently, 19 (90.5%) had PAMF serological profiles which were most compatible with an infection acquired in the distant past, and in 1 (4.8%), results were considered equivocal.

To evaluate the significance of detection of positive IgM test results without an established reference method, we included in our evaluation all sera with positive results in both tests for IgM antibodies. Results of the PAMF serological profile and/or results of follow-up specimens were available in 55 (80.9%) of 68 serum samples. Of these, 21 (38.2%) had PAMF serological profiles which were most compatible with an infection acquired recently, 21 (38.2%) had PAMF serological profiles which were most compatible with an infection acquired in the distant past, and in 13 (23.6%), results were considered equivocal.

Seroconverters. In each of the seven women who seroconverted during gestation, the IgG IMx became positive at the same time as did the dye test. In two patients, the IgM IMx result was equivocal at 11 and 15 weeks after infection, respectively, whereas the IgM ELISA was positive.

Reproducibility and precision. The results of precision tests (inter- and intra-assay) and reproducibility studies for negative and positive controls in the IgG and IgM IMx had coefficients of variation of less than 6.2 and 7.4%, respectively.

DISCUSSION

The data presented above reveal that there is excellent agreement between results obtained with the IgG IMx and the Sabin-Feldman dye test. In 10 (0.7%) of all 1,398 serum samples tested for IgG antibodies, the IgG IMx was more sensitive than the dye test; in 8 (0.6%) of the 1,398 serum samples, the dye test was more sensitive than the IgG IMx. The dye test is routinely performed in serum dilutions of 1:16 and higher, a policy established by the originators of the dye test in order to avoid considerable numbers of nonspecific cross-reactions at lower serum dilutions (5a). However, it has been recognized that IgG toxoplasma antibodies that reflect infection with the parasite may be present in titers of <1:16. Resolution of discrepancies between the 10 serum samples that were positive by the IgG IMx but negative by the dye test at a dilution of 1:16 revealed that 9 (90.0%) of these serum samples were positive by the dye test when repeated in lower dilutions. The direct agglutination test, performed in order to clarify whether the IgG titers in these nine serum samples represented specific reactions, revealed that six of eight of these serum samples (75%) had specific anti-toxoplasma IgG antibodies. Only one serum sample that tested positive in the IgG IMx was repeatedly negative when retested in the dye test, even at lower dilutions (from undiluted to 1:8). This serum sample was also negative in the direct agglutination test.

In the retrospective group, comparison of the results obtained with the IgM IMx and IgM ELISA revealed an agreement of 98.4%. This group comprised selected sera that had previously been shown to be either positive or negative for IgM antibodies in our IgM ELISA. Among the discrepancies observed in this group, the presence of a negative IgM IMx and a positive IgM ELISA was the most frequent (1% of sera in this group). Our attempt to resolve discrepancies suggested greater sensitivity for the IgM ELISA than for the IgM IMx in three of these serum samples (IgA ELISA positive or equivocal and acute pattern in the AC/HS). A specificity of the IgM IMx test lower than that for the IgM ELISA was suggested by the results for two serum samples (negative IgA ELISA and chronic pattern in the AC/HS). In the group of sera from pregnant women who seroconverted during gestation, we observed excellent agreement between the IgM ELISA and IgM IMx.

In the prospective group, composed of consecutive serum samples routinely tested in our laboratory, discrepancies between the IgM antibody tests were more frequent (4.7% of sera in this group). The presence of a positive IgM IMx and a negative IgM ELISA was the most frequent discrepancy (3.4% of sera in this group). Of these sera, 90.5% had a serological profile most compatible with an infection acquired in the distant past. A positive IgM ELISA and a negative IgM IMx was observed in 1.3% of sera. Of these, 62.5% had a serological profile most compatible with an infection acquired in the distant past. The discrepancies between the IgM ELISA and IgM IMx may in part be due to differences in the antigen preparations used in the two methods. In the IgM ELISA, the antigen preparation is a lysate of the tachyzoite form of the parasite that contains both cell membrane and cytoplasmic antigens. In contrast, in the IgM IMx assay, most of the reactivity of the antibodies is directed against the external surface antigens of the parasite.

Luyasu et al. recently evaluated the IgM IMx assay in a multicenter study (8). In their study, overall agreement between the IgM IMx and a capture IgM ELISA (Abbott Toxo IgM EIA) ranged from 99.4 to 99.6% (8). The excellent agreement between the two tests for IgM antibodies may have been due to the large numbers of randomly tested samples from blood donors or pregnant women and a smaller number of serum samples from patients with suspected toxoplasmosis. The sera investigated in their study appear to have been similar to those used in our retrospective group, which yielded similarly excellent agreement. In contrast, in our prospective study, the use of consecutive sera, including those sent from other laboratories for the resolution of inconclusive results, may have contributed to the higher numbers of discrepant results.

In order to investigate the significance of detection of IgM

antibodies, we also compared all positive results obtained with both the IgM IMx and the IgM ELISA with the PAMF serological profile. Of the sera with positive results in both tests, 38.5% came from patients with serological profiles which were most compatible with an infection acquired recently and 38.5% of sera came from patients with serological profiles which were most compatible with an infection acquired in the distant past. Despite the lack of an established reference method, detection of IgM antibodies by both tests strongly suggested the presence of IgM antibodies in these sera. The presence of IgM antibodies in the chronic stage of infection has been reported previously and is not rare (2, 5). Therefore, appropriate interpretation of positive IgM results is critical, especially for pregnant women. Our study clearly demonstrates that interpretation of positive results obtained in tests for IgM antibodies in most cases requires confirmatory testing with additional serological tests (e.g., tests for IgA and IgE antibodies and the AC/HS test). Even then, a serological diagnosis of acute infection often requires the demonstration of a significant rise in antibody titers in serial serum samples obtained at least 3 weeks apart (13, 19). This is of importance, since in the United States and most other countries, it is routine to base a decision on whether a pregnant woman has recently acquired the infection on results obtained from a single serum sample. In our experience, a positive result in an IgM antibody test has led to abortion in up to 20% of cases (3). Such abortions are even more tragic in view of the high rate of false-positive IgM tests (up to 60%) in many commercially available kits (12).

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REFERENCES

1. **Ashburn, D., R. Evans, L. J. Skinner, M. W. Chatterton, A. W. L. Joss, and D. O. Ho-Yen.** 1992. Comparison of relative uses of commercial assays for *Toxoplasma gondii* IgM antibodies. J. Clin. Pathol. **45:**483–486.
2. **Bobic, B., D. Sibalic, and O. Djurkovic-Djakovic.** 1991. High levels of IgM antibodies specific for *Toxoplasma gondii* in pregnancy 12 years after primary toxoplasma infection. Gynecol. Obstet. Invest. **31:**182–184.
3. **Candolfi, E., R. Ramirez, M.-P. Hadju, C. Shubert, and J. S. Remington.** 1994. The Vitek immunodiagnostic assay for detection of immunoglobulin M toxoplasma antibodies. Clin. Diagn. Lab. Immunol. **1:**401–405.
4. **Dannemann, B. R., W. C. Vaughan, P. Thulliez, and J. S. Remington.** 1990. Differential agglutination test for diagnosis of recently acquired infection with *Toxoplasma gondii*. J. Clin. Microbiol. **28:**1928–1933.
5. **Del Bono, V., A. Canessa, P. Bruzzi, M. A. Fiorelli, and A. Terragna.** 1989. Significance of specific immunoglobulin M in the chronological diagnosis of 38 cases of toxoplasmic lymphadenopathy. J. Clin. Microbiol. **27:**2133–2135.

5a. **Desmonts, G.** Personal communication.

6. **Desmonts, G., and J. S. Remington.** 1980. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. J. Clin. Microbiol. **11:**562–568.
7. **Ilstrup, D.** 1990. Statistical methods in microbiology. Clin. Microbiol. Rev. **3:**219–226.
8. **Luyasu, V., A. R. Robert, L. Schaefer, J. Macioszek, and Multicenter Study Group.** 1995. Multicenter evaluation of a new commercial assay for detection of immunoglobulin M antibodies to *Toxoplasma gondii*. Eur. J. Clin. Microbiol. Infect. Dis. **14:**787–793.
9. **Montoya, J. G., and J. S. Remington.** 1995. Studies on the serodiagnosis of toxoplasmic lymphadenitis. Clin. Infect. Dis. **20:**781–789.
10. **Naot, Y., and J. S. Remington.** 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. **142:**757–766.
11. **Petithory, J. C., I. Reiter-Owona, F. Berthelot, M. Milgram, J. De Loye, and E. Petersen.** 1996. Performance of European laboratories testing serum samples for *Toxoplasma gondii*. Eur. J. Clin. Microbiol. Infect. Dis. **15:**45–49.
12. **Remington, J. S., et al.** Unpublished observation.
13. **Remington, J. S., R. McLeod, and G. Desmont.** 1995. Toxoplasmosis, p. 140–243. *In* J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant, 1995. W. B. Saunders Co., Philadelphia.
14. **Sabin, A. B., and H. A. Feldman.** 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (toxoplasma). Science **108:**660–663.
15. **Stepick-Biek, P., P. Thulliez, F. G. Araujo, and J. S. Remington.** 1990. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J. Infect. Dis. **162:**270–273.
16. **Suzuki, Y., P. Thulliez, G. Desmonts, and J. S. Remington.** 1988. Antigen(s) responsible for immunoglobulin G responses specific for the acute stage of toxoplasma infection in humans. J. Clin. Microbiol. **18:**63–70.
17. **Verhofstede, C., L. Van Renterghem, and J. Plum.** 1989. Comparison of six commercial enzyme linked immunosorbent assays for detecting IgM antibodies against *Toxoplasma gondii*. J. Clin. Pathol. **42:**1285–1290.
18. **Wong, S. Y., M.-P. Hadju, R. Ramirez, P. Thulliez, R. McLeod, and J. S. Remington.** 1993. Role of specific immunoglobulin E in diagnosis of acute toxoplasma infection and toxoplasmosis. J. Clin. Microbiol. **31:**2952–2959.
19. **Wong, S. Y., and J. S. Remington.** 1994. Toxoplasmosis in pregnancy. Clin. Infect. Dis. **18:**853–862.