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False-Positive Results in Immunoglobulin M (IgM) Toxoplasma Antibody Tests and Importance of Confirmatory Testing: the Platelia Toxo IgM Test

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Although tests for detection of immunoglobulin M (IgM) toxoplasma antibodies have been reported to have a high degree of accuracy, it is well recognized by investigators in the United States and Europe that false-positive results may occur with many of these tests, at times to an alarming degree. Unfortunately, this information is not well documented in the literature. Studies on various toxoplasma IgM test kits are frequently flawed. The investigators often use reference tests which have not previously been carefully evaluated as well as sera that were not appropriate to answer the question of how often false-positive results might occur. We recently had the unique opportunity to evaluate the accuracy of the Platelia Toxo IgM test in 575 serum samples obtained during an outbreak of toxoplasmosis which occurred in 1995 in the Capital Regional District of British Columbia, Canada. When compared with results obtained in a reference IgM enzyme-linked immunosorbent assay (ELISA), the Platelia Toxo IgM test had a sensitivity of 99.4%, specificity of 49.2%, positive predictive value of 51.9%, negative predictive value of 99.3%, and an overall agreement of 67.0%. In an attempt to resolve discrepancies between these two tests, a serological profile (Sabin-Feldman dye test, IgA and IgE antibody tests, differential agglutination [AC/HS] test, and IgG avidity method) was performed. Of 153 serum samples that were positive in the Platelia Toxo IgM test and negative in the IgM ELISA, 71 (46.4%) were negative in the Sabin-Feldman dye test. Of the serum samples that were positive in the dye test, 77 (93.9%) had a serological profile most compatible with an infection acquired in the distant past. These results reveal high numbers of false-positive results in the Platelia Toxo IgM test and highlight the importance of appropriate evaluation of commercial tests that are currently being marketed. Our results also emphasize the importance of confirmatory testing to determine whether the results of an IgM antibody test reflect the likelihood of a recently acquired infection.

Serologic tests for detection of immunoglobulin M (IgM) antibodies are commonly performed for the diagnosis of acute acquired toxoplasma infection in the immunocompetent patient (21). Because IgM antibodies may persist for many months or even years following the acute infection (2, 6), their greatest value is in determining that a pregnant woman has not recently been infected. A negative result virtually rules out recently acquired infection unless sera are tested so early after the acute infection that an antibody response has not yet occurred or is not yet detectable.

Commercial kits for detection of toxoplasma IgM antibodies are increasingly being used. The majority of studies on IgM toxoplasma antibody tests have reported that they are highly accurate (3, 9, 11, 16, 19, 25, 26). Others, however, have reported high numbers of false-positive and false-negative results (1, 8, 13, 29, 30). Since interpretation of IgM toxoplasma antibody test results for the diagnosis of recently acquired infection 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 in an IgM antibody test (either due to tests which have low specificity or due to the presence of specific toxoplasma IgM antibodies in the chronic stage of the infection) can lead and has led to unnecessary concern and abortion (3, 22).

The Platelia Toxo IgM test is a widely used commercial method for detection of toxoplasma IgM antibodies. We recently had the unique opportunity to perform serological studies for toxoplasma antibodies on serum samples obtained during an outbreak of toxoplasmosis in the Capital Regional District of British Columbia, Canada (31). Results obtained in the Platelia Toxo IgM test were compared with results obtained in the IgM enzyme-linked immunosorbent assay (ELISA) developed in our laboratory and used by us and other reference laboratories for many years (17, 18). In an attempt to resolve discrepancies between these two tests, we analyzed results obtained by additional serological methods, including the Sabin-Feldman dye test (24), IgA (27) and IgE ELISAs (32), an IgE immunosorbent agglutination assay (ISAGA) (32), a differential agglutination (AC/HS) test (5), and the IgG avidity test (10, 14), as well as results obtained in follow-up serological tests.

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MATERIALS AND METHODS

A total of 575 serum samples collected from 475 individuals between May 1995 and March 1996 during an outbreak of toxoplasmosis in the Capital Regional District of British Columbia, Canada, were used for this study (31). The group of individuals tested comprised cases of acute toxoplasmosis (as defined by detection of toxoplasma-specific IgG and IgM antibodies and an acute pattern in the AC/HS test [14.9%]), their household contacts (4.0%), pregnant women (45.5%), and others (35.6%).

Sera were initially processed at the Provincial Laboratory, British Columbia Center of Disease Control, by using the Platelia Toxo IgM test (Sanofi Diagnostics Pasteur, Marne La Cornette, France) according to the manufacturer's instructions. Briefly, for evaluation of test results, two cutoff control sera provided by the manufacturer were included in each test run, and the mean value of the readings of the two cutoff sera was calculated. Values of the negative and positive controls provided by the manufacturer were compared with the mean cutoff value in order to validate each test run. Sera with values greater than or equal to the mean cutoff value were considered positive for the presence of IgM antibodies against *Toxoplasma gondii*. Values less than the cutoff value but greater than or equal to 80% of the cutoff value were considered equivocal. Sera with values less than 80% of the cutoff value were considered negative for detectable levels of IgM antibodies to *T. gondii*. Coefficients of variation for the intra-assay precision were 3.0, 5.0, and 19.6% for the strong-positive, weak-positive, and negative controls, respectively. Coefficients of variation for the interassay precision were 10.7, 17.9, and 42.3% for the strong-positive, weak-positive, and negative controls, respectively.

Between 29 April and 30 May 1995, all sera positive for toxoplasma IgG antibodies were referred to the Toxoplasma Serology Laboratory at the Palo Alto Medical Foundation (PAMF) for confirmation and additional testing; thereafter, only those sera with equivocal or positive results in the Platelia Toxo IgM test were referred. Confirmatory testing was performed as previously described by using a serological profile including the Sabin-Feldman dye test (24), double-sandwich IgM ELISA (18), IgA ELISA (27), IgE ISAGA (32), IgE ELISA (32), and the AC/HS test (5). The double-sandwich IgM ELISA method has been used by our laboratory and other reference laboratories for more than 15 years (17, 18). Briefly, for evaluation of test results, the following sera were used as controls: (i) a serum negative for IgM antibodies, (ii) a serum whose value in the IgM ELISA was in the mid-range, and (iii) a strongly positive serum. These sera were used to generate a standard curve on each plate. A low-positive control was included on each plate in order to validate the standard curve. The values of the negative and strongly positive controls were arbitrarily designated to be equal to 0 and 10 U, respectively. Values for each serum tested were derived from the standard curve. A result of $\geq$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. Coefficients of variation for the intra- and interplate precision were 12.2 and 16.5%, respectively. The following titers were considered positive, negative, or equivocal, respectively, in the various tests: IgA ELISA, $\geq$2.1, $\leq$1.4, 1.5 to 2.0; IgE ELISA, >1.8, $\leq$1.4, 1.5 to 1.8; IgE ISAGA, $\geq$4, $\leq$2, 3. The AC/HS test was interpreted as previously described (5) 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 (5, 28). The Sabin-Feldman dye test is considered positive at any titer. The starting dilution was 1:16.

We attempted to resolve discrepancies between results in the Platelia Toxo IgM test and the IgM ELISA. For this purpose, we used results obtained in the PAMF serological profile and the IgG avidity test (10, 14). Our results obtained in the PAMF serological profile were interpreted as being one of the following: (i) most compatible with an infection acquired recently, if confirmatory testing revealed results which were considered to reflect a recently acquired infection; (ii) most compatible with an infection acquired in the distant past, if confirmatory testing did not reveal an acute infection; and (iii) equivocal, if confirmatory testing revealed discordant results. Results obtained in follow-up specimens were also used in the interpretation. The IgG avidity measurement was performed with the *T. gondii* IgG avidity enzyme immunoassay (Labsystems, Helsinki, Finland) and interpreted according to the manufacturer's instructions (14).

Sensitivity was defined as the percentage of specimens positive in the IgM ELISA that were identified as positive by the Platelia Toxo IgM test. Specificity was defined as the percentage of specimens negative in the IgM ELISA that were identified as negative in the Platelia Toxo IgM test. The positive predictive value was defined as the probability that a positive Platelia Toxo IgM test result would be positive in the IgM ELISA. The negative predictive value was defined as the probability that a negative Platelia Toxo IgM test result would be negative in the IgM ELISA. Overall agreement was defined as the percentage of specimens that were positive or negative in the IgM ELISA and which gave the same positive and negative result in the Platelia Toxo IgM test (12).

RESULTS

Results of the comparison of the Platelia Toxo IgM test and the IgM ELISA are shown in Table 1. Of the 575 serum samples, 326 (56.7%) were positive in the Platelia Toxo IgM test and 175 (30.4%) were positive in the IgM ELISA. Of the 326 serum samples positive in the Platelia Toxo IgM test, 165 (50.6%) were positive and 153 (46.9%) were negative in the IgM ELISA. Of the 175 serum samples positive in the IgM ELISA, 165 (94.3%) were positive and 1 (0.6%) was negative in the Platelia Toxo IgM test. Results in the Platelia Toxo IgM test were equivocal in 98 (17.0%) serum samples, and in the IgM ELISA, results were equivocal in 11 (1.9%) serum samples. Sera with equivocal results were not used in the analysis of test accuracy. When compared with the IgM ELISA, the Platelia Toxo IgM test had a sensitivity of 99.4%, specificity of 49.2%, positive predictive value of 51.9%, negative predictive value of 99.3%, and an overall agreement of 67.0%.

Of the 154 serum samples with discrepant results, 153 were positive in the Platelia Toxo IgM test and negative in the IgM ELISA; one serum sample was negative in the Platelia Toxo IgM test and positive in the IgM ELISA. Of the 153 serum samples that were positive in the Platelia Toxo IgM test and negative in the IgM ELISA, 71 (46.4%) were negative and 82 (53.6%) were positive in the Sabin-Feldman dye test. Of the latter sera, 77 (93.9%) had a PAMF serological profile most compatible with an infection acquired in the distant past; one (1.2%) had a serological profile most compatible with an infection acquired recently. Resolution of discrepancies in four (4.9%) cases was not possible since the PAMF serological profile was interpreted as equivocal. One (0.7%) serum sample that was negative in the Platelia Toxo IgM test and positive in the IgM ELISA had a serological profile most compatible with an infection acquired in the distant past.

Results of the comparison of the IgG avidity test and the serological profile are shown in Table 2. Of the 153 serum samples positive in the Platelia Toxo IgM test and negative in the IgM ELISA, 64 (41.8%) were tested in the IgG avidity test (14). Testing for IgG avidity was not performed for 67 (43.8%) serum samples because specific IgG antibodies were not detectable; in an additional 22 (14.4%) cases, the quantity of serum was not sufficient. Of the 64 serum samples tested, 5 (7.8%) had low avidity IgG antibodies consistent with an infection acquired recently. In contrast, 49 (76.6%) had high

TABLE 1. Results in the Platelia Toxo IgM test and IgM ELISA

Comparison assay	Platelia Toxo IgM test result (%)[a]		
	Positive	Equivocal	Negative
IgM ELISA			
Positive	165 (28.7)	9 (1.6)	1 (0.2)
Equivocal	8 (1.4)	1 (0.2)	2 (0.3)
Negative	153 (26.6)	88 (15.3)	148 (25.7)

[a] $n = 575$.

TABLE 2. Results in the IgG avidity test and PAMF serological profile

Comparison assay	IgG avidity test result (%)[a]		
	Acute	Borderline	Chronic
PAMF serological profile			
Acute	0	1 (1.6)	0
Equivocal	1 (1.6)	1 (1.6)	2 (3.1)
Chronic	4 (6.3)	8 (12.5)	47 (73.4)

[a] $n = 64$.

TABLE 3. Reports on toxoplasma IgM ELISA in the literature

Study (reference)	Type of ELISA	Nos. and type of sera	Reference test	% Specificity	% Positive predictive value	Comments
Herbrink et al. (11)	Noncommercial and commercial	115 Sera from healthy laboratory personnel, 50 sera from 19 patients with acute infection	Immunoblot (IgM)	ND[a]	ND	Highly significant correlation (coefficient of 0.97–0.99) between test results
Rotmans et al. (23)	Noncommercial and immunoblot	763 Routine sera, not further specified	None	ND	ND	Poor agreement between ELISA tests, false-positive results in the indirect ELISA
Verhofstede et al. (30)	6 Commercial	28 IgM-positive sera, 53 IgM-negative sera from patients with different clinical conditions	Noncommercial ELISA, immunoblot (IgM)	87–100	ND	Only 3 of the 6 ELISA did not give false-positive results
Goullier-Fleuret and Picot (8)	Commercial	430 Sera, including 15 sera from patients with recent infections	Not specified, commercial ELISA and others	76.9–100	ND	Specificity of 57.1% without elimination of IgG antibodies using protein A
Joynson et al. (13)	5 Commercial	50 Selected sera (29 IgM positive), 177 routine sera	Noncommercial ELISA	79–100 (selected sera), 90–98 (routine sera)	ND	Accuracy of tests differs between selected and routine sera for each single test
Schaefer et al. (26)	Commercial	339 Sera, not further specified	Commercial ELISA, IgM ISAGA	98.7	ND	% Specificity was determined after resolution of discrepancies
Safford et al. (25)	Commercial	490 Sera, not further specified	Commercial ELISA, IgM ISAGA	98	ND	
Van Enk et al. (29)	3 Commercial	74 Selected sera (7 IgM positive, 12 IgG positive)	IFA[b]	52–100	ND	
Ashburn et al. (1)	7 Commercial	5 IgM-positive sera in defined dilutions	Noncommercial ELISA	85.7–100	ND	The authors suggest a combination of tests for diagnosis
Hall et al. (9)	Commercial	Not specified	Noncommercial ELISA, IgM ISAGA	93	ND	
Pelloux et al. (19)	Commercial	594 Routine sera, 39 seroconversion sera	Commercial ELISA, IgM ISAGA	97	ND	
Lappalainen et al. (14)	2 Commercial	44,181 Prenatal screening sera (3,178 IgG positive)	Commercial ELISA, immunoblot, and IgG avidity	ND	ND	Poor agreement between commercial ELISAs
Candolfi et al. (3)	Commercial	407 Selected sera (50% positive, 50% negative for IgM)	Noncommercial ELISA	93	93.6	% Specificity and positive predictive value were determined before resolution of discrepancies
Luyasu et al. (16)	Commercial	4,341 Routine sera including 179 selected IgM-positive sera	Commercial ELISA, IgM ISAGA	99.8	99.6–99.7	
Crouch (4)	Commercial	436 Routine sera	Commercial ELISA, IgM ISAGA	ND	ND	79.8% Agreement between test results
Petithory et al. (20)	Not specified (multicenter study)	5 Selected sera (4 negative, 1 positive for IgM)	Not specified	ND	ND	1–2% of laboratories reported borderline results for IgM negative sera
Gorgievski-Hrisoho et al. (7)	4 Commercial	146 Selected sera from 81 patients (varying in time after seroconversion)	Commercial ELISA, IFA	95	ND	Modified cutoff values and test combinations increased the specificity
Liesenfeld et al. (15)	Commercial	393 Selected sera (187 positive, 206 negative for IgM), 1,344 consecutive routine sera	Noncommercial ELISA	99.0 (selected sera), 95.9 (routine sera)	98 (selected sera), 74.7 (routine sera)	% Specificity and positive predictive value were determined before resolution of discrepancies

[a] ND, not done.
[b] IFA, immunofluorescence assay.

avidity IgG antibodies consistent with an infection acquired in the distant past; 10 serum samples (15.6%) were interpreted as borderline. There was excellent agreement between the IgG avidity test and the PAMF serological profile for sera of chronically infected patients. Of 49 serum samples that had high avidity antibodies, 47 (95.9%) had a PAMF serological profile consistent with an infection acquired in the distant past; in 2 (4.1%) cases, the PAMF serological profile was interpreted as equivocal. Of five serum samples that had low avidity IgG antibodies, four had a PAMF serological profile consistent with an infection acquired in the distant past; results obtained in follow-up sera were also consistent with an infection acquired in the distant past. In one case, the serological profile was interpreted as equivocal.

DISCUSSION

The data presented above reveal that there is poor agreement between results obtained with the Platelia Toxo IgM test and the IgM ELISA. The presence of a positive Platelia Toxo IgM test result and a negative IgM ELISA result was the most frequent discrepancy (26.6% of all sera). In addition, whereas the IgM ELISA was negative in the 121 serum samples that were IgG negative, the Platelia Toxo IgM was positive in 71 (58.7%) of these serum samples (data not shown). Follow-up sera from each of the individuals did not reveal seroconversion (data not shown). Thus, in these sera the Platelia Toxo IgM test result was clearly false positive. A similar discrepancy was noted in dye-test-positive sera. A total of 93.9% of these sera had a PAMF serological profile most compatible with an infection acquired in the distant past. Results obtained in the IgG avidity test revealed excellent agreement with the PAMF serological profile and the IgM ELISA for sera of chronically infected patients, and thus confirm our finding that the Platelia Toxo IgM test had a high number of false-positive results. The reasons for the discrepancies between the two IgM tests are unclear but include differences in antigen preparation and differences in the method and selection of sera used to establish the cutoff between positive and negative sera. The Platelia Toxo IgM test also produced markedly higher numbers of equivocal results compared with the IgM ELISA. According to the manufacturer's recommendations, equivocal results in the Platelia Toxo IgM do require the testing of a new serum collected 1 week later.

Despite the fact that false-positive results in tests for IgM toxoplasma antibodies have been described (1, 8, 13, 29, 30), the majority of studies on tests for IgM antibodies report high specificities (3, 9, 16, 19, 25, 26) (summarized in Table 3). Thus, one might conclude that such false-positive results are infrequent and therefore of little consequence. Unfortunately, in such studies, in order to evaluate a new diagnostic assay, it has been common practice to use as a reference test one that has not previously been carefully evaluated (Table 3). Since there is not an accepted "gold standard" for detection of toxoplasma IgM antibodies, parameters of test accuracy for these reference tests such as specificity and positive predictive value are ill defined. The inherent errors of these reference tests are frequently perpetuated and subsequently bias results of testing for accuracy in the new assays (12). The specificity and positive predictive value of new assays are directly related to the prevalence of negative and positive samples, respectively, as detected by the reference test. Therefore, the selection of sera used for evaluation markedly influences the accuracy of the test. To evaluate new assays for detection of IgM toxoplasma antibodies, either preselected sera (e.g., seroconversion sera) with known clearly positive or clearly negative results, routine sera with unknown reactivity (e.g., blood donors), or routine sera spiked with positive samples have been used (Table 3). Only rarely has the accuracy of new assays been evaluated in studies in which both large numbers of preselected sera and routine sera were used (Table 3). Using the latter approach, Joynson et al. (13) and our group (15) have shown that the accuracy of tests differs markedly depending on the use of selected or routine sera. Thus, because most reports in the literature do not reflect the overall accuracy of these tests, the actual numbers of false-positive results might be underestimated. Furthermore, the published literature on diagnostic tests tends to be biased towards studies yielding high accuracies.

The presence of toxoplasma IgM antibodies in the chronic stage of infection has previously been reported (2, 6) and complicates the appropriate interpretation of positive results, especially in pregnant women. In our study, 254 serum samples were obtained from pregnant women. Of these, 91 (35.8%) had a PAMF serological profile most consistent with an infection acquired in the distant past but a positive result in the Platelia Toxo IgM test. Therefore, confirmatory testing by either additional tests or the demonstration of a significant rise in antibody titers in serial serum samples obtained at least 3 weeks apart and run in parallel should be performed for sera with positive IgM test results (33). This is of utmost importance since in the United States and most other countries, it is routine to base the decision of whether a pregnant woman has recently acquired the infection on results obtained in a single serum sample. In our experience, pregnant women who are informed by their physician that they have a positive IgM test for toxoplasma antibodies will request abortion in up to 20% of cases (3, 22). This tragedy and that of such pregnant women who desire to continue their pregnancy but who will worry about its outcome for the remainder of gestation can be avoided by more careful evaluation of serology kits by regulatory agencies before they are released for use by the public and by more rigorous guidelines for the data to be submitted for such approval decisions.

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