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# Evaluation of Six Commercial Kits for Detection of Human Immunoglobulin M Antibodies to *Toxoplasma gondii*

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**As a result of reports received by the Food and Drug Administration (FDA) of false-positive results obtained with FDA-cleared in vitro diagnostic kits for the detection of *Toxoplasma*-specific human immunoglobulin M (IgM) antibodies, an FDA-sponsored evaluation of six kits was performed. A battery of 258 serum specimens, including 30 specimens drawn 1 to 5 months after initial *Toxoplasma* infection and 228 specimens from *Toxoplasma* IgG-positive individuals, *Toxoplasma* IgG-negative individuals, rheumatoid factor-positive persons, and persons determined to be *Toxoplasma* IgM positive by commercially available assays, was assembled, randomly assorted, and coded. The battery was tested at the FDA with six commercially available kits, at the Palo Alto Medical Foundation (PAMF) by the PAMF double-sandwich IgM enzyme-linked immunosorbent assay (PAMF IgM ELISA), and at the Centers for Disease Control and Prevention (CDC) by the CDC EIA IgM. The results of the PAMF IgM ELISA that were obtained with the battery were considered to be the "gold standard" for this study; specificity rates were computed by considering the PAMF results to be 100% specific. Sensitivity and specificity rates were found to be as follows: CDC EIA IgM, 100 and 99.1%, respectively; Abbott IMx Toxo IgM, version 1, 100 and 77.5%, respectively; Abbott IMx Toxo IgM, version 2, 93.3 and 97.3%, respectively; Abbott Toxo-M EIA, 100 and 84.2%, respectively; BioMérieux Vitek VIDAS Toxo IgM, 100 and 98.6%, respectively; BioWhittaker Toxocap-M, 100 and 95.9%, respectively; Gull Toxo IgM, 97 and 85.6%, respectively; and Sanofi Diagnostics Pasteur Platelia Toxo IgM, 100 and 96.8%, respectively. Although the extent of false-positive reactions with these kits cannot be calculated because the study was retrospective and sample choices were biased, the results may be useful as an indicator of the relative specificities of these kits.**

Because *Toxoplasma gondii* organisms or antigens are rarely detected in humans infected with *T. gondii*, antibody detection tests are used to indicate whether or not a person has been infected. The presence of *Toxoplasma*-specific immunoglobulin G (IgG) antibodies indicates infection at some time, but the level of IgG reactivity is not indicative of how recently the individual was infected. Determining when a pregnant woman became infected is extremely important so that appropriate measures can be taken to decrease fetal damage caused by transplacental infection. Detection of *Toxoplasma*-specific IgM antibodies is the most common method used throughout the world to attempt to determine when the infection occurred, but problems with the specificity of commercially available tests may result in the presentation of erroneous information to the physician and his or her patient, which may ultimately interfere with the physician's decisions related to clinical management.

In the United States, most *Toxoplasma*-specific IgM tests are performed with commercially available kits cleared by the Food and Drug Administration (FDA) for in vitro diagnostic use. Published evaluations of commercially available and in-house *Toxoplasma*-specific IgM assays have been summarized recently (4). As a result of additional reports received by the FDA of false-positive results obtained with FDA-cleared kits for the detection of *Toxoplasma*-specific human IgM antibodies, an FDA-sponsored evaluation of the six kits most used by U.S. laboratories was performed to determine the extent of specificity problems.

## MATERIALS AND METHODS

**Assays.** The commercially available kits chosen for inclusion in this study were used by 10 or more laboratories that participated in the 1995 College of American Pathology proficiency testing for *Toxoplasma* which is included in the Virology Antibody (VR3) survey. Although the Sigma SIA Toxoplasma IgM assay was used by 10 laboratories during 1995, the distributor changed manufacturers; consequently, the Sigma kit was excluded from the study. The following kits and lot numbers were used for this study: Abbott IMx Toxo IgM, version 1, lots 14292Q100, 16155Q100, and 21087Q100 and Abbott IMx Toxo IgM, version 2, lot 21881302 (Abbott Laboratories, Diagnostics Division, Abbott Park, Ill.); Abbott Toxo-M EIA lots 16561M300 and 20568M401 (Abbott Laboratories, Diagnostics Division, Abbott Park, Ill.); bioMérieux Vitek VIDAS Toxo IgM lot 618676A (bioMérieux Vitek, Hazelwood, Mo.); BioWhittaker Toxocap-M lot 6E1171 (Wampole Laboratories, Cranbury, N.J.); Gull Toxo IgM lot 6GMBHQ (Gull Laboratories, Salt Lake City, Utah); and Sanofi Diagnostics Pasteur Platelia Toxo IgM lot 6E143U (Sanofi Diagnostics Pasteur, Chaska, Minn.).

For this study, the double-sandwich IgM enzyme-linked immunosorbent assay (ELISA) performed by the Toxoplasma Serology Laboratory, Palo Alto Medical Foundation (PAMF), Palo Alto, Calif., (PAMF IgM ELISA) was designated the reference assay for determination of specificity because of the depth of experience and data accumulated with this assay (4, 6). Specimens with results of ≥2.0 are considered to be positive. The capture enzyme immunoassay (EIA) for IgM of the Centers for Disease Control and Prevention (CDC) (CDC EIA IgM) was performed by the Reference Immunodiagnostic Laboratory, Division of Parasitic Diseases, CDC, as described by Franco et al. (2), with some modifications. In brief, the assay consists of absorption of anti-human IgM to the microtiter plate,

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TABLE 1. Results of an evaluation of commercially available kits for detection of *Toxoplasma* IgM antibody

| Group | No. of specimens | No. of specimens called positive or no. of specimens called positive + no. called equivocal | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PAMF IgM ELISA | CDC EIA IgM | bioMérieux VIDAS Toxo IgM | Sanofi Platelia Toxo IgM | BioWhittaker Toxocap-M | Gull Toxo IgM | Abbott Toxo-M EIA | Abbott IMx Toxo IgM | |
| | | | | | | | | | Version 1 | Version 2 |
| A | 30 | 30 | 30 | 30 | 30 | 30 | 29 | 30 | 30 | 28 |
| B | 6 | 3 | 1 | 4 + 1 | 4 + 2 | 3 + 1 | 5 | 6 | 5 | 2 |
| C | 119 | 0 | 2 | 3 + 2 | 7 + 8 | 5 + 6 | 28 + 4 | 30 + 13 | 38 + 14 | 6 + 5 |
| D | 20 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 1 + 2 | 0 |
| E | 75 | 0 | 0 | 0 | 0 | 0 | 1 + 1 | 1 + 2 | 7 + 4 | 0 |
| F | 8 | 0 | 0 | 0 | 0 | 0 | 3 | 4 + 2 | 4 + 2 | 0 |

washing, incubation of diluted patient sample, washing, incubation of peroxidase-labeled *Toxoplasma* soluble antigen, washing, incubation with OPD substrate, and cessation of the reaction with sulfuric acid. Samples are tested in duplicate at a single dilution of 1:100 instead of serial dilutions. The result is calculated as follows: 10 × (mean absorbance for unknown sample − mean absorbance for negative calibrator)/(mean absorbance for positive calibrator − mean absorbance for negative calibrator) (12). The CDC positive calibrator has a value of 10.0 when tested in the PAMF IgM ELISA, as does the PAMF positive reference serum. A value of ≥2.0 was considered positive in the CDC EIA IgM.

The Sabin-Feldman dye test (11) was performed at PAMF, and the indirect immunofluorescence assay (IFA) (14) for IgG was performed at CDC.

**Serum specimens.** Specimens were chosen retrospectively from serum banks at CDC and PAMF for inclusion in a battery of 258 samples. Group A consisted of 30 plasma samples drawn 1 to 5 months after the onset of symptoms from 10 people infected in the 1977 *Toxoplasma* outbreak in Atlanta, Ga. (13); group B consisted of six serum specimens drawn 18 months postinfection from 6 additional patients in the 1977 Atlanta outbreak; group C consisted of 119 *Toxoplasma* IgG-positive (by either the Sabin-Feldman dye test or IFA) and IgM-negative (determined at PAMF or CDC) samples; group D consisted of serum samples from 20 individuals positive for rheumatoid factor (>10.9; Behring Diagnostics) at values ranging from 15 to 5,330; group E consisted of serum samples from 75 donors determined to be *Toxoplasma* IgG negative by IFA at CDC; and group F consisted of eight specimens reported by commercial laboratories to have positive *Toxoplasma* IgM results but found at CDC to have negative results for *Toxoplasma* IgG by IFA and *Toxoplasma* IgM by EIA.

All specimens were coded and randomly assorted at CDC to form the battery. Specimens were divided into nine replicate aliquots, and one replicate was assigned to each battery set. The battery sets were shipped frozen overnight to PAMF (one set) and the Winchester Engineering and Analytical Center (WEAC), FDA, Winchester, Mass. (six sets), and were stored at −20°C until they were tested. CDC kept one set for testing and one set in reserve.

**Test procedure.** All commercially available kits were tested at WEAC. To familiarize laboratory personnel with the kit procedure and to determine kit precision, a 3-day, six-run series of tests with kit controls and four positive serum specimens and two negative serum specimens from CDC was performed by the technologist by using modified recommendations presented in document EP5-T2 of the National Committee for Clinical Laboratory Standards (NCCLS) (7). The results were reviewed and were accepted or rejected before the next run. After this series of tests had been successfully completed, the technologist began the tests with the battery of specimens. A single battery set was designated for use with each kit; specimens were thawed for testing and were immediately refrozen after testing; all repeat tests were done with samples reserved from that battery. Each specimen was tested once (a single measurement) with each kit. All tests were performed by strictly following the instructions in the manufacturer's package insert. If the instructions provided with the kit suggested retesting of those specimens with equivocal or positive results, such retesting was performed. CDC and PAMF performed the tests according to their established laboratory procedures. All specimens with discrepant results (any specimen whose results differed from that of PAMF) were retested at WEAC with the appropriate kit to verify the initial result. If the first result was not duplicated by the results of the second test, the sample was tested a third time.

**Calculations.** All calculations are based on the initial result. Kit sensitivity was defined as the percentage of the 30 specimens from patients with toxoplasmosis that were determined to be positive as defined by each manufacturer. Specificity was defined as the percentage of the 222 specimens in groups C, D, E, and F (all found to be negative by the PAMF IgM ELISA) that were identified as negative by each kit. Indeterminate was defined as the percentage of the 222 negative specimens called equivocal (neither positive nor negative). Intra- and interassay precisions, as indicated by the coefficient of variation (CV), were calculated for each kit's positive and negative controls.

## RESULTS

The results obtained with the six commercially available kits for *Toxoplasma* IgM, the PAMF IgM ELISA, and the CDC EIA IgM are presented in Table 1. The sensitivities obtained with five kits (VIDAS, Platelia, BioWhittaker, Abbott EIA, and Abbott IMx, version 1), the CDC test, and the PAMF assay were 100%; reduced sensitivity was found with the Gull kit (97%) and with the Abbott IMx, version 2, kit (93%). Of the six serum specimens drawn 18 months after the onset of illness from patients with toxoplasmosis, at least two were called positive by all seven commercially available kits. Four kits (VIDAS, Platelia, BioWhittaker, and Abbott IMx, version 2) and the CDC assay had specificity rates of >95%, while three kits (Gull, Abbott EIA, and Abbott IMx, version 1) had specificity rates of 77 to 86%. The specimens with the highest rates of false positivity were those in group C (IgG-positive, IgM-negative samples). For rheumatoid factor-positive specimens, more positive results were observed with the BioWhittaker kit (4 of 20 group D samples) than with the other kits. In addition to the highest number of false-positive results, the Abbott IMx, version 1, kit had the greatest number of equivocal reactions. Sensitivity, specificity, and indeterminate rates are summarized in Table 2. Both intra- and interassay precisions for the positive and negative controls provided with the six kits are presented in Table 3.

## DISCUSSION

The battery of specimens was selected so that it included a large number of specimens that might be detected as false

TABLE 2. Sensitivity, specificity, and rates of equivocal results for commercially available kits for detection of *Toxoplasma* IgM[a]

| Kit | Sensitivity (%) | Specificity (%) | % Equivocal results |
|---|---|---|---|
| PAMF IgM ELISA | 100 | 100[b] | 0 |
| CDC EIA IgM | 100 | 99.1 | 0 |
| bioMérieux VIDAS Toxo IgM | 100 | 98.6 | 0.9 |
| Sanofi Platelia Toxo IgM | 100 | 96.8 | 3.6 |
| BioWhittaker Toxocap-M | 100 | 95.9 | 2.7 |
| Gull Toxo IgM | 97 | 85.6 | 2.3 |
| Abbott Toxo-M EIA | 100 | 84.2 | 7.7 |
| Abbott IMx Toxo IgM | | | |
| Version 1 | 100 | 77.5 | 9.0 |
| Version 2 | 93.3 | 97.3 | 2.3 |

[a] Sensitivity was calculated on the basis of results for 30 samples, and specificity was calculated on the basis of results for 222 samples.

[b] The results of the PAMF ELISA for IgM were used to calculate specificities.

TABLE 3. Summary of precision data for commercially available *Toxoplasma* IgM kits

| Type of control serum | Kit | Interassay[a] | | Intra-assay[b] | |
|---|---|---|---|---|---|
| | | No. of runs | CV range | No. of runs | CV |
| Positive | Abbott Toxo-M EIA | 6 | 2.1–6.3 | 14 | 4.9 |
| | Abbott IMx Toxo IgM, version 1 | 6 | 0.6–6.1 | 18 | 3.9 |
| | Abbott IMx Toxo IgM, version 2 | 6 | 0.7–4.9 | 29 | 6.7 |
| | BioWhittaker Toxocap-M | 6 | 0.6–2.7 | 11 | 10.6 |
| | Gull Toxo IgM | 8 | 2.2–22.2 | 13 | 13.2 |
| | Sanofi Platelia Toxo IgM | 8 | 0.8–5.1 | 13 | 13.4 |
| | bioMérieux VIDAS Toxo IgM | 6 | 1.8–11.3 | 18 | 4.8 |
| Negative | Abbott Toxo-M EIA | 6 | 3.4–7.5 | 14 | 8.2 |
| | Abbott IMx Toxo IgM, version 1 | 6 | 1.2–5.0 | 18 | 8.2 |
| | Abbott IMx Toxo IgM, version 2 | 6 | 1.5–15.0 | 29 | 11.5 |
| | BioWhittaker Toxocap-M | 6 | 3.1–13.4 | 11 | 12.7 |
| | Gull Toxo IgM | 8 | 0–114.6 | 13 | 116.4 |
| | Sanofi Platelia Toxo IgM | 8 | 1.9–42.8 | 13 | 27.8 |
| | bioMérieux VIDAS Toxo IgM | 6 | 0–6.9 | 18 | 8.0 |

[a] Three replicates per run.
[b] One sample per run.

positive and not so that it was representative of specimens from the general population; therefore, the specificity rates calculated in this study cannot be extrapolated to those for the general population. If these kits had been used to test an unselected series of specimens submitted for routine screening for *Toxoplasma*, much lower rates of false positivity would be expected (see package inserts) (1, 3–5). However, this study's results should be taken into consideration when interpreting a positive result obtained with any of the six kits: for example, a positive result with the bioMérieux VIDAS kit (specificity, 98.6%) appears to be more reliable than a positive result with the Abbott EIA, the Abbott IMx, version 1, or the Gull kit (specificities, 84.2, 77.5, and 85.6%, respectively).

Most false-positive reactions were concentrated in group C, which were from IgG-positive and IgM-negative individuals. However, as the reactors in group F indicate, false-positive IgM reactions also may occur for patients who do not have *Toxoplasma* IgG antibodies. False-positive IgM reactions were detected by only two kits (BioWhittaker and Abbott IMx, version 1) for patients who were rheumatoid factor positive. Of the assays used in this study, all assays except the Abbott IMx and Gull assays are configured in the capture IgM format, which theoretically provides complete elimination of all isotypes other than IgM in the initial step of the assay. The Gull test relies on preabsorption of the specimen to eliminate the patient's IgG before testing, while the Abbott IMx assay allows all immunoglobulin isotypes to bind to the antigen but then probes the antigen-antibody complex for only IgM reactions by testing with a labeled anti-IgM reagent.

To obtain an estimate of kit sensitivity, 30 specimens from symptomatic patients with toxoplasmosis were included in the battery. Because they were symptomatic, the time interval between the onset of symptoms and the date that the specimen was drawn could be calculated. However, these patients cannot be considered typical patients with toxoplasmosis because they were infected during a unique outbreak situation and were all symptomatic, unlike most individuals who are infected with *T. gondii*. Indeed, all specimens reacted at high levels (≥5.0) in the PAMF IgM ELISA. No specimens were included in the battery that reacted in the range of ≥2.0 to 5.0 by the PAMF assay, which may also be considered suggestive of recent infection. Consequently, detection of all 30 samples as positive by most of the kits in this study only indicates that most of the kits consistently detect high-titer specimens. Also, the samples were all obtained 1 month or more after the onset of symptoms; therefore, the efficiency of detection of very early infections could not be determined. A much larger sample of specimens with results over all titer ranges should be evaluated to more accurately determine kit sensitivity.

An additional indication of kit sensitivity is observed with the group B samples, drawn 18 months after the onset of symptoms from individuals infected with *T. gondii* (Table 1). All tests detected at least one sample as IgM positive. Ideally, these tests would detect IgM antibodies during the initial 6 to 9 months after infection and not thereafter to allow for a better estimation of when an individual is initially infected. This is particularly important so that appropriate counseling can be given to pregnant women when indicated.

Table 2 summarizes the sensitivity and specificity calculations as well as the percentage of equivocal reactors. These were included as additional indicators of the usefulness of each kit. Equivocal results usually require additional testing by another assay and/or testing of a second blood specimen from the patient. Consequently, those assays with the best combination of sensitivity, specificity, and few equivocal results are the most cost-efficient. For example, in this study, the VIDAS assay had

TABLE 4. Guide to general interpretation of *Toxoplasma* serology results obtained by commercially available assays

| IgG result | IgM result | Report or interpretation for humans except infants |
|---|---|---|
| Negative | Negative | No serological evidence of infection with *T. gondii* |
| Negative | Equivocal | Possible early acute infection or false-positive IgM reaction; obtain a new specimen for IgG and IgM testing; if the result for new specimen remains the same, the patient is probably not infected with *T. gondii* |
| Negative | Positive | Possible acute infection or false-positive IgM result; obtain a new specimen for IgG and IgM testing; if results for the second specimen remain the same, the IgM reaction is probably false positive |
| Equivocal | Negative | Indeterminate; obtain a new specimen for testing or retest this specimen for IgG in a different assay |
| Equivocal | Equivocal | Indeterminate; obtain a new specimen for both IgG and IgM testing |
| Equivocal | Positive | Possible acute infection with *T. gondii*; obtain a new specimen for IgG and IgM testing; if results for the new specimen remain the same or the result for IgG becomes positive, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing |
| Positive | Negative | Infected with *T. gondii* for more than 1 year |
| Positive | Equivocal | Probably infected with *T. gondii* for more than 1 year and false-positive IgM reaction; obtain a new specimen for IgM testing; if results for the new specimen remain the same, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing |
| Positive | Positive | Possible recent infection within the last 12 months; send the specimen to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing |

1.4% false-positive and 0.9% equivocal results, so only 2.3% of the specimens would need further testing, whereas the Abbott IMx, version 1, assay had 22.5% false-positive and 9% equivocal results, for a total of 32% of the specimens that would need additional testing.

Although the results found in this study indicate good specificity for the Sanofi Platelia assay, recent findings in the laboratory of one of the authors (J.S.R.) indicated poor specificity (49.2%) with other lots of the kit from the same manufacturer (4), perhaps indicating variability among kit lots. A clinical laboratory must not rely on the manufacturer to ensure a continuing supply of comparable lots of reagents but must institute its own verification procedures for each kit lot as well as performing an initial evaluation (8–10). The specimens included in a panel for evaluation purposes should be carefully selected to include some with a high probability of cross-reactions (groups C and D samples), as well as those with a normal probability of cross-reactions (group E), in addition to samples that are truly positive for *Toxoplasma* IgM (group A) and that have a range of reactivities.

Two additional shortcomings of this study are that (i) only one kit lot of a kit from each manufacturer instead of multiple lots obtained over time was used to test the battery and (ii) the kits were bought directly from the manufacturers, who knew that the kits would be used for this study. It is possible that the manufacturer chose the best available kit lot as opposed to a random kit lot that a hospital laboratory might purchase. It is hoped that these two points will be addressed in a future study.

Precision rates are generally presented as the assay precision rate but actually reflect the combination of the technologist's precision and that of the assay. The Abbott EIA and the BioWhittaker, Gull, and Sanofi assays are manual assays that require several manipulations (preparation of patient specimen dilutions, addition of specimen dilutions to the matrix, several plate washings, addition of conjugate, and preparation and addition of substrate) by the technician, whereas with the Abbott IMx and the VIDAS assays, the addition of a measured amount of undiluted patient serum to the test cassette is the only manual procedure required. One would therefore expect improved precision with the automated assays as opposed to the manual assays. Interassay CVs for the positive control were less than 6.3% for all commercially available kits tested except the VIDAS (automated) and Gull (manual) kits. For the VIDAS assay, the interassay CVs for five runs were under 3.4%, but the interassay CV was 11.3% for one run. For the Gull assay, interassay CVs for eight runs were 10.5, 4.3, 4.4, 16.5, 2.2, 22.2, 12.6, and 7.3%, respectively, indicating a higher degree of assay and/or technical imprecision. Intra-assay precision for the positive control for the two automated assays was less than 6.7%, while it varied from 4.9 to 13.4% for the manual assays. Inter- and intra-assay precisions for the negative control were less for the automated assays than for the manual assays.

Interpretation of *Toxoplasma* serology results is exceedingly difficult (i) because most assays are not based on results for patients documented to be infected with parasites because identification of infected patients by detection of the organism or its antigens occurs only rarely; (ii) because of a lack of standardization for reporting of the results; and (iii) because of various rates of sensitivity and specificity for the many assays available. None of the commercially available assays described here have been evaluated by the manufacturers for their usefulness with fetal or infant specimens and are not cleared by FDA for this purpose. If acute infection is suspected, a patient sample should be tested for the presence of *Toxoplasma*-specific IgG and IgM antibodies. Table 4 presents a guide to general interpretation of serology results for adults obtained by commercially available assays in the United States. The laboratory technician must assume the responsibility of understanding the problems inherent with these assays and should give the requesting health care provider all the information possible about the usefulness of the assays used to determine the patient's antibody status. Health care providers must take the responsibility to educate themselves about the problems with the tests and therapy options before counseling a patient who has positive IgM results.

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