

Detection of Immunoglobulin G (IgG) and IgM Antibodies to *Toxoplasma gondii*: Evaluation of Four Commercial Immunoassay Systems

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A comparative evaluation of the following commercial immunoassays for the determination of antibodies to *Toxoplasma gondii* was performed: Behring Diagnostics OPUS Toxo G and Toxo M, Abbott Diagnostics IMX Toxo-IgG 2.0 and Toxo-IgM, Sanofi Diagnostics Pasteur Platelia Toxo IgG and Toxo IgM, and bioMérieux Vitek VIDAS Toxo IgG and IgM. Of 676 specimens that were tested for *Toxoplasma*-specific immunoglobulin G (IgG) antibodies, 26% were reactive by all methods while 8% displayed some discrepancy. Of 718 specimens that were tested for *Toxoplasma*-specific IgM antibodies, 3% were reactive by all methods while 10% displayed some discrepancy. Analysis of discrepant specimens revealed performance shortcomings with all IgM-specific assays. The impact of such shortcomings is magnified in a population with a low prevalence of toxoplasmosis.

Toxoplasmosis is a common and generally benign disease in immunocompetent persons caused by *Toxoplasma gondii*, which is an intestinal coccidian parasite of felines. From 3.2 to 13.3% of young adults in the United States are seropositive for *Toxoplasma* antibodies (12). In immunocompromised individuals (especially congenitally infected infants, organ transplant recipients, and individuals with AIDS), toxoplasmosis may cause life-threatening complications. Laboratory diagnosis relies on detection and quantitation over time of specific *Toxoplasma* immunoglobulin G (IgG) and IgM antibodies. Detection and measurement of IgG-specific antibodies is rarely problematic, and good sensitivity and specificity have been achieved by a variety of methods (2, 5, 6, 8, 11). Detection of IgM antibodies is more problematic because of the reported low degree of test specificity and the clinical implications of a false-positive result, which can lead to unnecessary therapeutic intervention (1, 4, 7). In our study population, which was heavily weighted to include immunocompromised patients (transplant recipients, patients receiving chemotherapy, and AIDS patients), a number were repeatedly reactive in *Toxoplasma* IgM antibody studies in the absence of IgG seroconversion or other evidence of active toxoplasmosis. The goals for the present study included a comparative evaluation of performance characteristics for four newer immunoassay systems to detect IgG and IgM antibodies to *T. gondii*.

The evaluation included blood specimens referred to the clinical laboratories of the University of Washington, Harborview Medical Center, and the Veterans Administration Medical Center between October 1995 and October 1996. A total of 676 specimens were referred for routine *Toxoplasma* IgG antibody testing, and 718 specimens were referred for routine *Toxoplasma* IgM antibody testing. Of these, 47 specimens were acquired prior to this time period and were included because they were found to be positive for *Toxoplasma* IgG and/or IgM

antibodies by previous routine testing. Blood specimens (red-top tubes) were clotted and centrifuged, and serum samples were examined for signs of hemolysis or lipemia. Archived (frozen at -20°C) sera were centrifuged prior to evaluation. The sera were reevaluated after thawing and centrifugation, and hemolytic, lipemic, or bacterially contaminated specimens were excluded. Acceptable sera were then tested for IgG and/or IgM anti-*Toxoplasma* antibodies. The commercial immunoassay systems evaluated included Behring Diagnostics OPUS Toxo G and Toxo M (OPUS Toxo M was a premarket evaluation), Abbott Diagnostics IMX Toxo-IgG 2.0 and Toxo-IgM, Sanofi Diagnostics Pasteur Platelia Toxo IgG and Toxo IgM, and bioMérieux Vitek VIDAS Toxo IgG and IgM. OPUS Toxo M, Sanofi Diagnostics Pasteur Platelia Toxo IgM, and bioMérieux Vitek VIDAS Toxo IgM use IgM capture methodology for detection of *Toxoplasma* IgM antibodies. Sanofi Diagnostics Pasteur Platelia Toxo IgG and Toxo IgM are enzyme immunoassays configured in the microtiter plate format. The other immunoassay systems that were evaluated are fully automated. Analysis of all immunoassay systems was performed according to the manufacturers' protocols. Discrepant IgG specimens and all IgM-positive or equivocal specimens from any assay were evaluated further by the Sabin-Feldman dye test (10), sensitized direct agglutination (13), and/or the IgM immunosorbent agglutination assay (3) as confirmatory tests. Confirmatory testing by the aforementioned methods was kindly performed by bioMérieux (France) and/or by the Institut de Puériculture de Paris (Paris, France).

The results for *Toxoplasma* IgG and IgM antibody testing among the evaluated immunoassay systems, including discrepant results, are shown in Table 1. Overall agreement rates among the four immunoassay systems were 91.7% for *Toxoplasma* IgG and 89.8% for *Toxoplasma* IgM. For 29 of the 56 discrepant *Toxoplasma* IgG specimens, confirmatory testing by the Sabin-Feldman dye test and sensitized direct agglutination was performed. The remaining discrepant *Toxoplasma* IgG specimens had insufficient volume for further testing. For 75 of the 93 reactive or discrepant *Toxoplasma* IgM specimens, confirmatory testing was performed by the IgM immunosorbent

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TABLE 1. Agreement and discrepancies among the immunoassay systems for detection of *Toxoplasma* IgG and IgM antibodies

<i>Toxoplasma</i> antibody	No. of specimens (% of total)			
	Tested by all four methods	Nonreactive by all four methods	Reactive by all four methods	Discrepant among methods
IgG	676 (100.0)	447 (66.1)	173 (25.6)	56 (8.3)
IgM	718 (100.0)	625 (87.0)	20 (2.8)	73 (10.2)

agglutination assay and the Sabin-Feldman dye test. The remaining reactive or discrepant *Toxoplasma* IgM specimens had insufficient volume for further testing. The results of confirmatory testing for *Toxoplasma* IgG and IgM antibodies on the discrepant results are shown for each system in Table 2. For calculations of overall test sensitivities and specificities, we regarded the remaining *Toxoplasma* IgG or IgM test results as confirmed negative or *Toxoplasma* IgG test results as confirmed positive when all four immunoassay systems showed similar results. These numbers were combined with the results obtained from confirmatory testing (Table 2), and the resolved sensitivities, specificities, and positive and negative predictive values for the different immunoassay systems are shown in Table 3.

Approximately 28% of our specimens were confirmed to be positive for *Toxoplasma* IgG antibodies, and 4.2% were confirmed to be positive for *Toxoplasma* IgM antibodies. The specimens included in our study were submitted over a 1-year period by clinicians from the three participating hospitals in Seattle for routine *Toxoplasma* antibody testing. Other tertiary-care centers in the United States have comparable patient populations and seropositivity rates. Agreement among the four immunoassay systems was very good for the detection of *Toxoplasma* IgG antibodies, and all evaluated systems showed similar sensitivities and specificities (Table 3). Similar findings for *Toxoplasma* IgG antibody determination have been reported by other investigators (2, 6, 8, 9). These results demonstrate that the four immunoassay systems for detection of anti-*Toxoplasma* IgG antibodies give comparable results and can be readily adapted by clinical laboratories for screening purposes. The four different immunoassay systems showed more variation in performance with regard to *Toxoplasma* IgM antibody detection. The most-sensitive test for *Toxoplasma* IgM antibody detection was the OPUS Toxo M test, which also provided the lowest positive predictive value (45.8%). The most specific test for IgM antibody detection was the VIDAS Toxo IgM test. This test also provided the highest positive

TABLE 2. Evaluation of the 29 discrepant *Toxoplasma* IgG specimens and 75 discrepant *Toxoplasma* IgM specimens after confirmatory testing

Test system	No. (%)			
	True positive	True negative	False positive	False negative
OPUS Toxo G	7 (24)	12 (41)	8 (28)	2 (7)
IMX Toxo-IgG 2.0	7 (24)	12 (41)	8 (28)	2 (7)
Platelia Toxo IgG	1 (3)	14 (48)	6 (21)	8 (28)
VIDAS Toxo IgG	4 (14)	19 (66)	1 (3)	5 (17)
OPUS Toxo M	27 (36)	13 (17)	32 (43)	3 (4)
IMX Toxo-IgM	23 (31)	33 (44)	12 (16)	7 (9)
Platelia Toxo IgM	22 (29)	37 (49)	8 (11)	8 (11)
VIDAS Toxo IgM	21 (28)	40 (53)	5 (7)	9 (12)

TABLE 3. Resolved sensitivities, specificities, and positive and negative predictive values of the evaluated immunoassay systems

Test system	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
OPUS Toxo G	98.9	98.3	95.7	99.6
IMX Toxo-IgG 2.0	98.9	98.3	95.7	99.6
Platelia Toxo IgG	95.6	98.7	96.7	98.3
VIDAS Toxo IgG	97.3	99.8	99.4	98.9
OPUS Toxo M	90.0	95.2	45.8	99.5
IMX Toxo-IgM	76.7	98.2	65.7	98.9
Platelia Toxo IgM	73.3	98.8	73.3	98.8
VIDAS Toxo IgM	70.0	99.3	80.8	98.7

predictive value (80.8%). In determining the usefulness of one of the four evaluated immunoassay systems for a specific laboratory setting, it is important to consider the prevalence of toxoplasmosis in the population being tested. The prevalence of acute seroconversion to this parasite in the United States is very low. It may be assumed that most positive *Toxoplasma* IgM antibody test results in a setting with a low prevalence of acute seroconversion are false positives (7). Any positive *Toxoplasma* IgM antibody result usually leads to additional workup of the patient, with repeated testing as well as possible therapeutic intervention. Interventions may include the use of potentially toxic antiprotozoal therapy in an immunocompromised individual or therapeutic abortion in a pregnant female because of concerns about fetal infection. Therefore, it is desirable to achieve a balance between sensitivity and specificity that is appropriate for the patient population being evaluated. Careful assessment of this patient population, the number of specimens being analyzed annually, per test costs, the run size, and the limitations of each immunoassay system must be considered when determining which is the most appropriate test for a particular clinical setting.

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