

Evaluation of *Toxoplasma gondii* Immunoglobulin G (IgG) and IgM Assays Incorporating the New Vidia Analyzer System[∇]

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The new Vidia system is a fully automated system based on chemiluminescence and antigen bound to magnetic microparticles, which allows a fast measurement of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM levels. The analytical performances of the Vidia Toxo IgG and IgM assays were compared with those of the automated Vidas, AxSYM, and Liaison Toxo IgG and IgM assays. The comparative evaluation was performed utilizing 204 frozen sera belonging to 166 subjects and 201 fresh sera collected from 198 subjects. For the Vidia Toxo IgG system, the sensitivities were 100% in both the retrospective and prospective studies, and specificities were 98.39% in the retrospective study and 100% in the prospective study, respectively. The sensitivities of the other three Toxo IgG assays were 100%, and the specificities ranged from 96.77% to 100%. For the Vidia Toxo IgM assay, the sensitivity and specificity were 100% in both the retrospective and prospective studies. The overall sensitivities and specificities of the other three Toxo IgM assays ranged from 80% to 100% and from 99.44% to 100%, respectively. In our study, the Vidia system revealed excellent sensitivity (100% for both IgG and IgM assays) and good specificity (99.25% for IgG and 100% for IgM assays).

Toxoplasma gondii is an obligate intracellular protozoan responsible for common parasitic infections throughout the world in a wide range of hosts including humans (2, 6, 8).

In immunocompetent subjects, acute infection is usually asymptomatic or characterized by mild, nonspecific clinical symptoms (e.g., cold or light case of the flu), and spontaneous recovery is the rule (8). However, in pregnant women, acute infection by *T. gondii* may result in congenital disease, causing abortion or severe damages to the fetus at birth or later in life (1), and/or sequelae that may be prevented or reduced by early treatment (10, 13). Toxoplasmic chorioretinitis can be seen in congenitally or perinatally acquired disease as a result of acute infection or reactivation (8).

In immunocompromised patients such as patients infected by human immunodeficiency virus and bone marrow or organ transplant recipients, toxoplasmosis almost always happens as a result of a reactivation of a chronic infection, and it can be a life-threatening condition presenting with encephalitis, chorioretinitis, pneumonitis, or multiorgan involvement (8).

During infection, IgM antibodies may appear earlier and decline more rapidly than IgG antibodies and are frequently the first class of antibodies detected after primary infection (7). Moreover, they may persist for months or years after infection, and only negative results in the vast majority of cases exclude acute infection (1).

The diagnosis of recently acquired toxoplasmosis is usu-

ally based on the detection of specific IgM antibodies, seroconversion, or a fourfold or greater rise in the titer of *T. gondii*-specific IgG antibodies (12). As seroconversion and a rise in IgG titers are seldom demonstrable, the detection of *T. gondii*-specific IgM antibodies has been the most frequently used serological marker for diagnosing acute infection. However, the interpretation of serological tests is complicated by the prevalence of high IgG titers among healthy individuals and the persistence of specific IgM in some people (12).

Thus, the serological diagnosis of toxoplasmosis is very complex and has been discussed extensively in the literature (8, 11); diagnosis is especially difficult for immunocompromised individuals, who have a reduced or absent production of antibodies, and pregnant women.

The absence of IgG antibodies before or early in pregnancy allows the identification of women at risk of acquiring infection, and the presence of IgG allows the identification of immunocompromised patients at risk for the reactivation of a latent infection.

Many serological tests for the detection of *T. gondii*-specific immunoglobulin are commercially available, and the enzyme-linked immunosorbent assay is one of the easiest tests to perform (3, 5, 7, 9). Originally, immunoassays were performed in a microtiter plate format using an enzyme-based color detection method, but more recently, they have been made available for autoanalyzers that use a range of detection methods including chemiluminescence and immunofluorescence.

The purpose of this study was to evaluate the analytical performance of the new automated Vidia Toxo IgG and IgM (bioMérieux, Marcy l'Etoile, France) immunoassays and compare the results with those of the Vidas Toxo IgG and IgM

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(bioMérieux), AxSYM Toxo IgG and IgM (Abbott Laboratories, Abbott Park, IL), and Liaison Toxo IgG and IgM (DiaSorin, Saluggia, Italy) assays.

MATERIALS AND METHODS

Samples. (i) Retrospective study for *Toxoplasma* IgG and IgM. Frozen aliquots from 204 sera from 166 subjects (median age of 39 years, ranging from 1 month to 85 years) sent to the Section of Microbiology of the University Hospital of Parma (Italy) for the serological diagnosis of toxoplasmosis during 2005 and 2006 were analyzed in the retrospective study. Before testing, samples were thawed and clarified by centrifugation at $2,000 \times g$ for 10 min. Thawed samples were kept at 4°C until use (no more than 5 days) before refreezing. In particular, the 204 samples were collected from 104 pregnant women, 55 immunocompromised patients, 27 children/infants, 11 blood donors, 6 pregnant human immunodeficiency virus-infected women, and 1 healthy adult.

(ii) Prospective study for *Toxoplasma* IgG and IgM. From July to September 2006, a total of 201 samples from 198 subjects (median age of 43 years, ranging from 3 months to 86 years) prospectively obtained from the laboratory of diagnostic *Toxoplasma* IgG and IgM testing were stored at 4°C until use (no more than 5 days) and then frozen at -20°C. In particular, the 201 samples were collected from 139 pregnant women, 38 healthy adults, 16 immunocompromised patients, 4 children, and 4 blood donors.

Tests. Each sample from both retrospective and prospective studies was tested in parallel or within 24 h at the same time by the four fully automated enzyme immunoassays. Testing was carried out according to the manufacturers' instructions.

The Vidas, AxSYM, and Liaison systems were described elsewhere previously (3, 7, 9).

The Vidas system, which is routinely utilized in our laboratory, uses a solid-phase receptacle coated with membrane and cytoplasmic *Toxoplasma* antigen (RH Sabin strain) and an anti-human IgG or IgM conjugate and 4-methylumbelliferyl phosphate as a substrate (7). For the Vidas Toxo IgG and IgM tests, positive results are defined as value of ≥ 8 international units (IU)/ml and index values of ≥ 0.65 , equivocal results range from 4 to 8 IU/ml and from index values of 0.55 to 0.65, and negative results are defined as < 4 IU/ml and index values of < 0.55 , respectively.

The AxSYM system uses *T. gondii*-coated microparticles as the solid phase for IgG detection and microparticles coated with IgG to human IgM for IgM detection; bound *T. gondii* IgG or IgM is detected with anti-human IgG or IgM conjugate and 4-methylumbelliferyl phosphate as a substrate (3). For AxSYM Toxo IgG and IgM tests, positive results are defined as values of ≥ 3 IU/ml and index values of ≥ 0.800 , equivocal results range from 2 to 3 IU/ml and from index values of 0.600 to 0.799, and negative results are defined as < 2 IU/ml and index values of < 0.600 , respectively.

The Liaison immunoassay uses magnetic microparticles coated with inactivated *T. gondii* (RH strain) and monoclonal antibodies labeled with an isoluminol derivative (9). For Liaison Toxo IgG and IgM tests, positive results are defined as a value of ≥ 8 IU/ml and an index value of ≥ 0.800 index, equivocal results range from 6 to 8 IU/ml and from index values of 0.600 to 0.799, and negative results are defined as < 6 IU/ml and index values of < 0.600 , respectively.

The Vidia system combines a two-step enzyme immunoassay method with paramagnetic microparticles and a final chemiluminescence detection step. The Vidia system has not previously been described fully.

In brief, in the Vidia Toxo IgG assay, 10 μ l of sample is used: specific IgG in the sample is bound to the beads, unbound material is removed by washing, and an alkaline phosphatase-labeled monoclonal anti-human IgG antibody is added. After incubation, a wash step removes the unbound conjugate. During the final detection step, the substrate is transformed into a luminescent product by the conjugate alkaline phosphatase. The intensity of the luminescence increases according to the quantity of *Toxoplasma* IgG in the sample. The instrument measures the relative luminescence value. Results are automatically analyzed and calculated by the instrument using the factory master calibration curve data, which are stored in the instrument and are expressed in IU/ml. Depending on the test value, positive results are defined as a value of ≥ 5 IU/ml, equivocal results range from 3 to 5 IU/ml, and negative results are defined as a value of < 3 IU/ml.

In the Vidia Toxo IgM assay, 10 μ l of sample is used: *Toxoplasma* IgM in the sample is bound to the beads, unbound material is removed by washing, and an inactivated Toxo antigen is added, which is itself revealed by an alkaline phosphatase-labeled anti-Toxo monoclonal antibody. After incubation, a wash step removes the unbound conjugate. During the final detection step, the substrate is transformed into a luminescent product by the conjugate alkaline phosphatase.

TABLE 1. Details of equivocal results or nonresolved samples

Study	No. of equivocal and/or nonresolved samples	No. of samples with equivocal results for:			
		Vidia	Vidas	AxSYM	Liaison
Retrospective study					
<i>Toxoplasma</i> IgG	20	8	13	5	5
<i>Toxoplasma</i> IgM	12	9	6	4	0
Prospective study					
<i>Toxoplasma</i> IgG	9	6	3	2	0
<i>Toxoplasma</i> IgM	4	2	2	2	0

The intensity of the luminescence increases according to the quantity of *Toxoplasma* IgM in the sample and is measured as the relative luminescence value. Results are automatically calculated by the instrument using the factory master data, which are stored in the instrument, and are expressed as an index. No international standard is available for testing of *Toxoplasma* IgM. The positive threshold is established and maintained using collection sera with known statuses. Positive results are defined as index values of ≥ 1.00 , equivocal results range from index values of 0.68 to 1.00, and negative results are defined as index values of < 0.68 .

Analysis of results. The samples for which at least one equivocal result has been found with any of the methods used were excluded from the analysis.

The "status" of samples (positive or negative) was defined on the basis of concordant results obtained by at least three of the four assays compared in this study.

Discrepancies were resolved by considering individual clinical data, when available, and/or by repetition of the test.

The analytical sensitivity and specificity of each assay were determined by expressing the results obtained as a ratio of samples with appropriately assigned positive or negative status, respectively. The accuracy of each assay was calculated based on the percentage of correct results obtained by the test under evaluation.

RESULTS

The sensitivity and specificity of the Vidia Toxo IgG and IgM tests were determined using all sera, excluding results obtained from 29 (7.1%) sera for IgG and 16 (3.9%) sera for IgM, for which at least one equivocal result or not-resolved status was observed (Table 1).

***Toxoplasma* IgG.** Sensitivities were 100% for the Vidia Toxo IgG assay and for the other assays in both the retrospective and prospective studies. Specificities were 96.77% for the AxSYM assay (which gave four false-positive results), 98.39% for the Vidia Toxo IgG assay (which gave two false-positive results), and 100% for the Vidas and Liaison tests in the retrospective study. No false-positive results were resolved by repeating the discordant assays. In the prospective study, specificities were 100% for all assays (Table 2).

***Toxoplasma* IgM.** In the retrospective study, the sensitivities were 100% for the Vidia Toxo IgM assay as well as for the other methods except for the AxSYM *Toxoplasma* IgM test, which had a sensitivity of 83.33% (2 false-negative results out of 10 positive samples). In the prospective study, the sensitivities were 100% for the Vidia and Vidas tests and 80% for the AxSYM and Liaison tests (one false-negative result out of four positive samples).

The specificities in the retrospective study were 100% for the Vidia Toxo IgM, Vidas Toxo IgM, and Liaison Toxo IgM tests. The AxSYM assay gave one false-positive result, with a spec-

TABLE 2. *Toxoplasma* IgG performance

Study	No. of samples	No. of equivocal and/or nonresolved samples	No. of negative results	No. of positive results	No. of false-positive results	No. of false-negative results	% Sensitivity (95% confidence interval)	% Specificity (95% confidence interval)	% Accuracy (95% confidence interval)
Retrospective <i>Toxoplasma</i> IgG	204	20							
Status ^a			124	60					
Vidia			122	60	2	0	100.0 (93.75–100.0)	98.39 (94.18–99.57)	98.91 (96.03–99.70)
Vidas			124	60	0	0	100.0 (93.75–100.0)	100.0 (96.88–100.0)	100.0 (97.87–100.0)
AxSYM			120	60	4	0	100.0 (93.75–100.0)	96.77 (91.86–98.76)	97.82 (94.45–99.16)
Liaison			124	60	0	0	100.0 (93.75–100.0)	100.0 (96.88–100.0)	100.0 (97.87–100.0)
Prospective <i>Toxoplasma</i> IgG	201	9							
Status ^a			141	51					
Vidia			141	51	0	0	100.0 (92.73–100.0)	100.0 (97.24–100.0)	100.0 (97.95–100.0)
Vidas			141	51	0	0	100.0 (92.73–100.0)	100.0 (97.24–100.0)	100.0 (97.95–100.0)
AxSYM			141	51	0	0	100.0 (92.73–100.0)	100.0 (97.24–100.0)	100.0 (97.95–100.0)
Liaison			141	51	0	0	100.0 (92.73–100.0)	100.0 (97.24–100.0)	100.0 (97.95–100.0)
Total <i>Toxoplasma</i> IgG	405	29							
Status ^a			265	111					
Vidia			263	111	2	0	100.0 (96.52–100.0)	99.25 (97.23–99.8)	99.46 (98.03–99.85)
Vidas			265	111	0	0	100.0 (96.52–100.0)	100.0 (98.51–100.0)	100.0 (98.94–100.0)
AxSYM			261	111	4	0	100.0 (96.52–100.0)	98.49 (96.12–99.42)	98.93 (97.24–99.59)
Liaison			265	111	0	0	100.0 (96.52–100.0)	100.0 (98.51–100.0)	100.0 (98.94–100.0)

^a The “status” of samples (positive or negative) was defined on the basis of concordant results obtained by at least three of the four assays compared in this study.

ificity of 99.44%. In the prospective study, specificities were 100% for all the assays (Table 3).

The overall sensitivities considering the results of both retrospective and prospective studies were 100% for all the IgG assays, while they were 100% for the Vidia and Vidas IgM assays and 82.35% and 94.12% for the AxSYM and Liaison IgM assays, respectively.

DISCUSSION

The serological diagnosis of *T. gondii* infections usually does not present any interpretative problems for immunocompetent subjects, but there are four groups of individuals for which the diagnosis of toxoplasmosis is most critical: pregnant women who acquire the infection during gestation, fetuses and new-

TABLE 3. *Toxoplasma* IgM performance

Study	No. of samples	No. of equivocal and/or nonresolved samples	No. of negative results	No. of positive results	No. of false-positive results	No. of false-negative results	% Sensitivity (95% confidence interval)	% Specificity (95% confidence interval)	% Accuracy (95% confidence interval)
Retrospective <i>Toxoplasma</i> IgM study	204	12							
Status ^a			180	12					
Vidia			180	12	0	0	100.0 (75.0–100.0)	100.0 (97.83–100.0)	100.0 (98.97–100.0)
Vidas			180	12	0	0	100.0 (75.0–100.0)	100.0 (97.83–100.0)	100.0 (98.97–100.0)
AxSYM			179	10	1	2	83.33 (54.59–95.41)	99.44 (96.83–99.9)	98.43 (95.42–99.47)
Liaison			180	12	0	0	100.0 (75.0–100.0)	100.0 (97.83–100.0)	100.0 (98.97–100.0)
Prospective <i>Toxoplasma</i> IgM	201	4							
Status ^a			192	5					
Vidia			192	5	0	0	100.0 (55.56–100.0)	100.0 (97.96–100.0)	100.0 (98.00–100.0)
Vidas			192	5	0	0	100.0 (55.56–100.0)	100.0 (97.96–100.0)	100.0 (98.00–100.0)
AxSYM			192	4	0	1	80.0 (36.85–96.48)	100.0 (97.96–100.0)	99.49 (97.10–99.91)
Liaison			192	4	0	1	80.0 (36.85–96.48)	100.0 (97.96–100.0)	99.49 (97.10–99.91)
Total <i>Toxoplasma</i> IgM	405	16							
Status ^a			0	0	372	17			
Vidia			0	0	372	17	0	0	100.0 (80.95–100.0)
Vidas			0	0	372	17	0	0	100.0 (80.95–100.0)
AxSYM			0	0	371	14	1	3	82.35 (58.45–93.93)
Liaison			0	0	372	16	0	1	94.12 (72.45–98.98)

^a The “status” of samples (positive or negative) was defined on the basis of concordant results obtained by at least three of the four assays compared in this study.

borns who are congenitally infected, immunocompromised patients, and those with chorioretinitis (11).

In this study, the performances of four commercially available automated immunoassays for the detection of *Toxoplasma* IgG and IgM were evaluated using 405 collected serum samples, 204 frozen samples and 201 fresh samples, obtained by laboratory diagnostic *Toxoplasma* IgG and IgM testing.

For *Toxoplasma* IgG, the best overall accuracy was obtained with the Vidas and Liaison assays (100%). The Vidia and AxSYM assay accuracies were 99.46% and 98.93%, respectively. For *Toxoplasma* IgM, the best overall accuracy was obtained with the Vidia and Vidas assays (100%), followed by the Liaison (99.74%) and AxSYM (98.97%) assays.

Except for the Vidas assays, which gave no false-negative or false-positive results, Vidia assays reported no false-negative and the fewest false-positive results (two false-positive results for IgG) with respect to the AxSYM (five false positive for IgG and three false negative for IgM) and Liaison (one false negative for IgM) tests.

No previous data are available for Vidia assays for either IgG or IgM, whereas in a previous comparative study, the Liaison, AxSYM, and Vidas Toxo IgG and IgM assays were evaluated (9): the Liaison IgG assay showed agreements of 91% and 100% with the AxSYM IgG and Vidas IgG assays; the Liaison IgM assay showed agreements of 95% and 97% with the AxSYM IgM and Vidas IgM assays. The sensitivity and specificity of the Liaison system (combined IgG and IgM results compared to the Sabin-Feldman dye test, which measures total immunoglobulin) were 99.3% and 96.8%, respectively (9). The sensitivities of the Vidas assay were 97.3% for IgG and 70% for IgM, and specificities were 99.8% for IgG and 99.3% for IgM, as reported previously (4). In our study, for *Toxoplasma* IgG, all methods gave the best sensitivity (100%), and all the methods, except the Vidia (99.25%) and AxSYM (98.49%) tests, gave the best specificity (100%). For *Toxoplasma* IgM, all methods gave the best sensitivity (100%), except for the AxSYM (82.35%) and Liaison (94.12%) systems, and all methods gave the best specificity (100%), except for the AxSYM (99.73%) system.

In conclusion, in our study, the Vidia system revealed excellent sensitivity (100% for both IgG and IgM assays) and good specificity (99.25% for IgG and 100% for IgM assays).

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