Comparative evaluation of AxSYM, VIDAS and VIDIA toxoplasmosis reagent performance in a high seroprevalence Latin American country

Avaliação comparativa entre o desempenho dos reagentes para toxoplasmose AxSYM, VIDAS e VIDIA em um país latinoamericano com alta soroprevalência

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ABSTRACT

Aims: The purpose of this study was to compare the performance of three automated immunoassays for the detection of IgM and IgG *Toxoplasma gondii* antibodies using sera of pregnant women living in Colombia, a Latin American country with a high seroprevalence. **Methods:** A total of 905 sera were tested for IgM antibodies and 914 for IgG antibodies with AxSYM, VIDAS and VIDIA immunoassays. Discrepancies were resolved by using the dye test for IgG antibodies, and the ISAGA test for IgM. **Results:** The overall agreement between AxSYM, VIDAS and VIDIA assays was excellent for detection of IgG and IgM antibodies, and discrepancies were relatively rare (3.6% and 5.5% of sera for IgG and IgM antibodies, respectively). The performance of the three immunoassays was similar for the detection of IgG antibodies with high sensitivity (100.00% for VIDIA, 99.59% for VIDAS, 99.38% for AxSYM) and specificity (99.04% for VIDIA, 98.82% for AxSYM, 98.57% for VIDAS). The specificity for IgM antibodies was excellent for the three immunassays (99.88% for VIDIA, 99.76% for AxSYM and VIDAS). The sensitivity of the detection of IgM antibodies was higher with VIDIA (95.12%) than with VIDAS (76.74%) and AxSYM (61.90%) assays. The correlation between IgG titers was limited between AxSYM and VIDAS assays and between AxSYM and VIDIA assays, but was excellent between VIDIA and VIDAS assays. **Conclusions:** Our study performed with Latin American sera confirmed the excellent specificity of AxSYM, VIDAS and VIDIA assays for the detection of IgG and IgM antibodies already reported in other countries. The sensitivity of the detection of IgG antibodies was slightly higher with VIDIA than with VIDAS and AxSYM assays. The sensitivity of the detection of IgM antibodies was higher with VIDIA than with VIDAS and AxSYM assays.

Keywords: TOXOPLASMOSIS/epidemiology; TOXOPLASMOSIS/immunology; TOXOPLASMOSIS/diagnosis; SEROLOGIC TESTS; ANTIBODY AVIDITY; ENZYME-LINKED IMMUNOSORBENT ASSAY; LATIN AMERICA; FEMALE; PREGNANT WOMEN; COMPARATIVE STUDIES.

INTRODUCTION

Toxoplasmosis is caused by the unicellular protozoan parasite *Toxoplasma gondii* (*T. gondii*). In immunocompetent subjects, primary infection is usually asymptomatic or associated with self-limited symptoms such as fever, malaise, and cervical lymphadenopathy. Infection acquired during pregnancy is frequently associated with transmission of *T. gondii* to the fetus, resulting in congenital disease. In immunocompromised patients, reactivation of latent infection can cause life-threatening complications.[1,2]

Infection with the parasite is widespread throughout the world, but the seroprevalence varies considerably between countries (from less than

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10% to more than 90%) and population groups. Toxoplasmosis is more prevalent in some regions of Europe, in Latin America, and sub-Saharan Africa, than in Asia, North America, and Oceania.[3-5] The impact of toxoplasmosis on public health is especially high in Latin America. Severe clinical forms with visceral localization, mainly pulmonary involvement, have been described after a primary infection in immunocompetent subjects in French Guiana.[6,7] A high prevalence of ocular toxoplasmosis has also been reported in Brazil.[8,9] *T. gondii* strains isolated from these subjects exhibited an atypical genotype, highly divergent from European and North American lineages.[6,10]

The diagnosis of recently acquired toxoplasmosis is usually based on the detection of specific immunoglobulin (Ig) M antibodies, in conjunction with seroconversion or a significant increase in specific IgG antibodies. Methods for the detection of anti-*T. gondii* antibodies include the Sabin-Feldman dye test, enzyme immunoassay (EIA), immunosorbent agglutination assay (ISAGA), IgA and IgE antibody tests, the differential agglutination test, IgG avidity test, enzyme-linked immunofiltration assay (ELIFA) and immunoblotting (IB).[1,11,12] Immunoassays are among the easiest tests to perform, and automated systems, well adapted to large-scale screening, have been developed.

The purpose of this study was to compare the performance of different automated immunoassays, AxSYM® TOXO IgG and IgM (Abbott Laboratories, Chicago, Ill, USA), VIDAS® TOXO IgG and IgM (bioMérieux, Marcy l'Etoile, France) and VIDIA® TOXO IgG and IgM (bioMérieux) using sera collected from pregnant women living in a Latin American country with a high seroprevalence of toxoplamosis.

METHODS

Samples and patients

This multicenter study was performed from October 2008 to May 2009 on frozen serum samples (n=920) provided by the Fundacion Valle del Lili, Cali, Colombia and collected during a previous epidemiological study led by the Palo Alto Medical Foundation Toxoplasmosis Serology Laboratory, Stanford University School of Medicine and Fundacion Valle del Lili.[5] The eligible population was pregnant women whose serum had been obtained from clinical blood samples already drawn for routine prenatal care in the ambulatory setting or for obstetric-related care in hospitalized patients.

Ethics

Ethics approvals were obtained for the epidemiological study, from the institutional review boards in the healthcare institutions in Cali, as well as in the Stanford University and Palo Alto Medical Foundation Research Institute. Informed consent was obtained from all patients referred for the Cali study. To preserve patient anonymity, a code was attributed by the evaluators to each sample tested. Investigators outside Fundacion Valle del Lili only had access to these codes and did not have access to patients' names.

Assays

All sera had already been tested for IgG and IgM *T. gondii* antibodies at the Fundacion Valle del Lili as part of the Cali study mentioned above, using the routine laboratory technique, VIDAS® (bioMérieux, Marcy l'Etoile, France) and then stored at -20°C. In addition, all IgG-positive/IgM-positive sera were tested using the VIDAS TOXO IgG Avidity assay. For the current study, aliquots of sera were thawed and clarified by centrifugation at 2000 g for 10 minutes, prior to testing at the Laboratoires Réunis, Junglinster, Luxembourg using the VIDIA® Toxo IgG and IgM (bioMérieux, Marcy l'Etoile, France) and the AxSYM Toxo IgG and IgM (Abbott Laboratories, Chicago, Ill, USA) immunoassays between October 2008 and January 2009. Analysis was performed according to manufacturers' recommendations. Any discrepant results were resolved using the dye test for IgG and ISAGA for IgM (Toxo-ISAGA®; bioMérieux, Marcy l'Etoile, France), performed as previously described[13,14] at the Institut de Puériculture de Paris (Paris, France).

The AxSYM, VIDAS and VIDIA systems have been described elsewhere.[15-19] The AxSYM system is based on microparticle enzyme immunoassay (MEIA) technology with a final fluorescence detection.[15,16] The AxSYM Toxo IgG and IgM assays use microparticles coated with cell-cultured *T. gondii* antigen (RH strain) as the solid phase. Bound anti-Toxoplasma IgG or IgM are detected with alkaline phosphatase-labeled anti-human IgG or IgM conjugate and 4-methylumbelliferyl phosphate is used as a substrate.

The VIDAS system combines a two-step enzyme immunoassay for IgG and an immunocapture method for IgM with a final fluorescence detection (ELFA).[17,18] The VIDAS TOXO IgG assay uses a solid-phase receptacle coated with membrane and cytoplasmic *T. gondii* antigen (RH Sabin strain grown in mice). The conjugate uses alkaline phosphatase-labeled mouse monoclonal anti-human IgG antibody

and 4-methylumbelliferyl phosphate as a substrate. The VIDAS TOXO IgM assay uses a solid-phase receptacle coated with polyclonal anti-human IgM antibody (goat). The conjugate contains an immune complex of *T. gondii* antigen and alkaline phosphatase-labeled anti-P30 mouse monoclonal antibody and 4-methylumbelliferyl phosphate is used as a substrate.

The VIDIA system combines a two-step immunoassay for IgG and immunocapture method for IgM with paramagnetic microparticles and a final chemiluminescence detection.[19] For VIDIA TOXO IgG, specific anti-*T. gondii* IgG are captured by the immunopurified cell-cultured tachyzoite antigen (RH Sabin strain) present on the magnetic particles, and then detected by an alkaline phosphatase-labelled monoclonal anti-human IgG antibody. For VIDIA TOXO IgM, the anti-*T. gondii* IgM in the sample are captured by the monoclonal anti-human IgM antibody present on the magnetic particles. Bound anti-*T. gondii* IgM are detected by the immune complex of *T. gondii* antigen (RH Sabin strain grown in mice) and alkaline phosphatase-labeled anti-P30 mouse monoclonal antibody. During the final detection step, the substrate (4-methylumbelliferyl phosphate) is transformed into a luminescent product by the conjugate alkaline phosphatase.

Data analysis

Only sera tested using all three techniques were analyzed. A concordance analysis was performed between AxSYM and the other two techniques, and discrepant results were resolved for each case using the dye test and ISAGA technique. After resolution of discrepant results and non-inclusion of equivocal results, the 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 correlation analysis was performed on all samples with an antibody titer >0 IU/mL and <300 IU/mL. A Passing-Bablok plot was used to analyze the agreement between the different techniques using Analyse-It® method validation software (Analyse-it Software, Ltd., Leeds, United Kingdom).[20]

RESULTS

Comparison between AxSYM, VIDAS and VIDIA immunoassays

Of the 920 sera, 905 were tested for IgM *T. gondii* antibodies and 914 for IgG *T. gondii* antibodies using AxSYM, VIDAS and VIDIA immunoassays (Table 1). A total of 476 sera (52.1%) were positive for IgG antibodies, and 22 sera (2.4%) were positive for IgM antibodies with the three immunoassays. A total of 402 sera (44.0%) were negative for IgG antibodies, and 831 sera (91.8%) were negative for IgM antibodies with the three immunoassays. Discrepancies were observed in 33 sera (3.6%) for IgG antibodies and 50 sera (5.5%) for IgM antibodies. These discrepant sera were assessed for IgG antibodies by using the dye test and for IgM antibodies by using the ISAGA test. Using the dye test, nine sera were positive and 24 negative for IgG antibodies. Using the ISAGA test, 28 sera were positive, three equivocal, and 19 negative for IgM antibodies. Combining the concordant results of the three methods and the results of the reference tests for discrepant sera, 485 sera (476+9) (53.1%) were considered as true positive for IgG antibodies, and 50 sera (22+28) (5.5%) as true positive for IgM antibodies.

Comparison between AxSYM and VIDAS assays

The agreement between AxSYM and VIDAS assays was 99.6% for positive and 98.8% for negative IgG results (Table 2). For IgM, agreement was 81.5% for positive and 98.9% for negative results (Table 3). Major discrepancies (positive result with one test, and negative result with the other test) were observed for seven IgG results and for 14 IgM results. Using the dye test, results of the AxSYM assay were confirmed for three sera and those of VIDAS for four sera. Using the ISAGA test, results of the AxSYM assay were confirmed for three sera and results of VIDAS for nine sera, two sera being equivocal.

Comparison between AxSYM and VIDIA assays

The agreement between AxSYM and VIDIA assays was 99.6% for positive and 99.0% for negative IgG results (Table 2). For IgM, agreement was 92.9% for positive and 98.7% for negative results (Table 3). Major discrepancies were observed for six IgG results, and for 13 IgM results. Using the dye test, results of the AxSYM assay were confirmed for three sera and those of VIDIA for three sera as well. Using the ISAGA test, results of the AxSYM assay were confirmed for one serum, and results of the VIDIA for 12 sera.

Table 1. Agreement and discrepancy between AxSYM, VIDAS and VIDIA immunoassays for the detection of *T. gondii* IgG and IgM antibodies

T. gondii antibody	Agreement between three assays			Discrepancies between assays	Resolution of discrepancies*		
	Positive	Equivocal	Negative		Positive	Equivocal	Negative
IgG	476	3	402	33	9	0	24
IgM	22	2	831	50	28	3	19

* Discrepancies were resolved by using the dye test for IgG and ISAGA test for IgM.

Table 2. Comparison of AxSYM, VIDAS and VIDIA assays for *T. gondii* specific IgG antibody detection

Assay	Result	Titer (IU/mL)	AxSYM IgG			Total
			Positive (≥3)	Equivocal (2-3)	Negative (<2)	
VIDAS IgG	Positive	≥8	480	3	5*	488
	Equivocal	4-8	1	4	4	9
	Negative	<4	2†	3	412	417
	Total		483	10	421	914
	Agreement		99.6%		98.8%	
VIDIA IgG	Positive	≥5	477	3	4‡	484
	Equivocal	3-5	4	4	9	17
	Negative	<3	2§	3	408	413
	Total		483	10	421	914
	Agreement		99.6%		99.0%	

* 3 negative sera and 2 positive sera with the dye test; † 2 negative sera with the dye test; ‡ 3 negative sera and 1 positive serum with the dye test; § 2 negative sera with the dye test

Table 3. Comparison of AxSYM, VIDAS and VIDIA assays for *T. gondii* specific IgM antibody detection

Assay	Result	Index	AxSYM IgM			Total
			Positive (≥0.800)	Equivocal (0.600-0.799)	Negative (<0.600)	
VIDAS IgM	Positive	≥0.65	22	6	9*	37
	Equivocal	0.55-0.65	2	2	6	10
	Negative	<0.55	5†	11	842	858
	Total		29	19	857	905
	Agreement		81.5%		98.9%	
VIDIA IgM	Positive	≥1.00	26	4	11‡	41
	Equivocal	0.68-1.00	1	7	13	21
	Negative	<0.68	2§	8	833	843
	Total		29	19	857	905
	Agreement		92.9%		98.7%	

* 1 negative serum, 7 positive sera and 1 equivocal serum with the ISAGA test; † 2 negative sera, 2 positive sera and 1 equivocal serum with the ISAGA test; ‡ 1 negative serum and 10 positive sera with the ISAGA test; § 2 negative sera with the ISAGA test

Sensitivity and specificity of AxSYM, VIDAS and VIDIA assays

IgM antibodies

After resolution of discrepant results, the VIDIA assay presented the highest sensitivity (95.12%), followed by VIDAS (76.74%) and AxSYM (61.90%) assays. The specificity was high for the three immunoassays, i.e. 99.88% for VIDIA, 99.76% for both AxSYM and VIDAS (Table 4).

IgG antibodies

After resolution of discrepant results, the sensitivity was high for the three immunoassays, i.e. 100.00% for VIDIA, 99.59% for VIDAS, and 99.38% for AxSYM. The specificity was also high for the three immunoassays, i.e. 99.04% for VIDIA, 98.82% for AxSYM and 98.57% for VIDAS (Table 4).

Table 4. Resolved sensitivities, specificities for AxSYM, VIDAS and VIDIA immunoassays for *T. gondii* specific antibody detection

Assay	Sensitivity*		Specificity†	
	%	[95% CI]	%	[95% CI]
AxSYM IgG	99.38%	[98.15%; 99.79%]	98.82%	[97.22%; 99.50%]
VIDAS IgG	99.59%	[98.47%; 99.89%]	98.57%	[96.88%; 99.36%]
VIDIA IgG	100.00%	[99.17%; 100.00%]	99.04%	[97.52%; 99,63%]
AxSYM IgM	61.90%	[46.51%; 75.23%]	99.76%	[99.12%; 99.94%]
VIDAS IgM	76.74%	[61.94%; 87.00%]	99.76%	[99.13%; 99.94%]
VIDIA IgM	95.12%	[83.54%; 98.68%]	99.88%	[99.31%; 99.98%]

* Sensitivity = true positive/(true positive + false negative)
† Specificity = true negative/(true negative + false positive)

Comparison between IgG antibody titers obtained with AxSYM, VIDAS and VIDIA

AxSYM versus VIDAS

The relationship between the titers measured with the VIDAS and AxSYM assays was not linear. However, by performing Passing and Bablok linear regression, it was possible to estimate an antibody titer ratio which was approximately 3.5 times higher with the VIDAS assay than with AxSYM (Figure 1a).

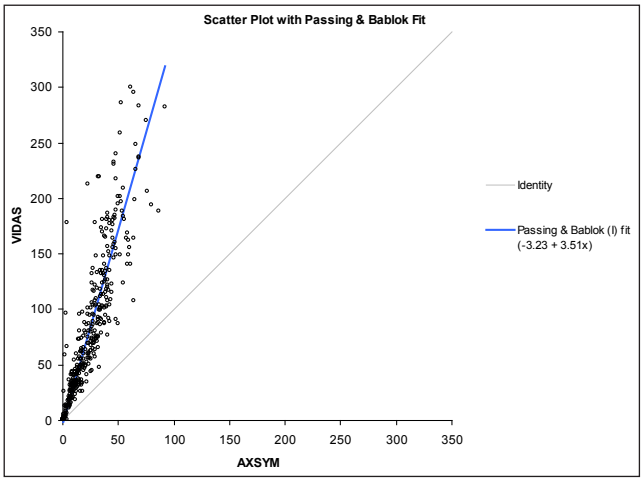

Figure 1a. Scatter plot of IgG titers measured with VIDAS and AxSYM assays.

AxSYM versus VIDIA

The relationship between the titers measured with the VIDIA and AxSYM assays was not linear. However, by performing Passing and Bablok linear regression, it was possible to estimate an antibody titer ratio which was approximately 2.8 times higher with the VIDIA assay than with AxSYM (Figure 1b).

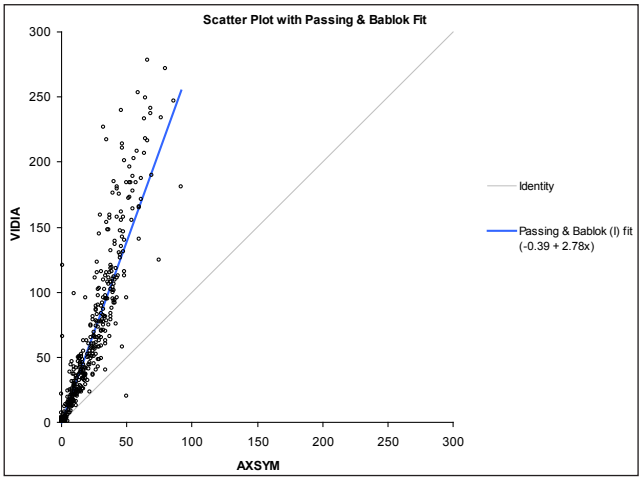

Figure 1b. Scatter plot of IgG titers measured with VIDIA and AxSYM assays.

VIDAS versus VIDIA

The relationship between the titers measured with the VIDAS and VIDIA assays was not linear. However, by performing Passing and Bablok linear regression, it was possible to estimate an antibody titer ratio which was approximately 1.1 times higher with the VIDAS assay than with VIDIA, showing that IgG antibody titers were very close between the two assays (Figure 1c).

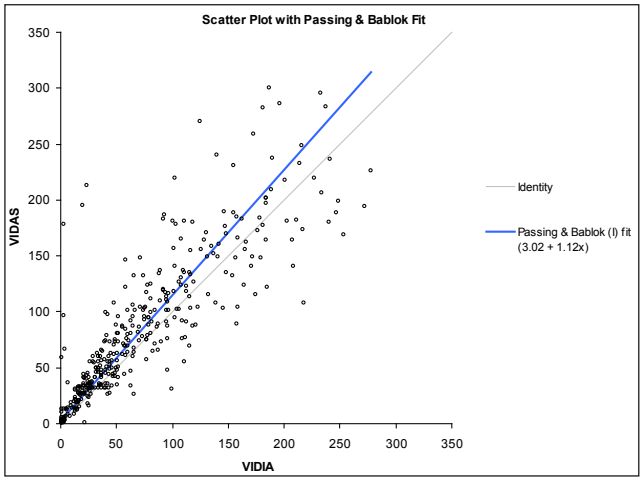

Figure 1c. Scatter plot of IgG titers measured with VIDAS and VIDIA assays

DISCUSSION

The objective of this study was to evaluate three commercially available automated toxoplasmosis immunoassays using sera collected in a Latin American country with a high *T. gondii* seroprevalence. Overall, 53.1% of the sera tested were considered true positive for IgG *T. gondii* antibodies, indicating a high seroprevalence in pregnant women. These results are consistent with the reported seroprevalence of 45.8% in this population.[5]

Most studies comparing different automated immunoassays have been conducted in European or North American countries. In these regions, *T. gondii* has a low genetic diversity, and most strains belong to three main clonal and closely related lineages, namely types I, II and III. Several studies suggest that "exotic" or "atypical" strains circulate in South America, Asia, and Africa. These strains are characterized by frequent genetic exchange generating a variety of recombinants, and a higher genetic diversity, especially in the Amazonian area. In immunocompetent subjects, atypical genotypes have been associated with severe clinical signs and multi-organ failure in French Guiana, and a higher occurrence of ocular toxoplamosis in Brazil.[6,7,9,10,21,22]

The overall agreement between AxSYM, VIDAS and VIDIA assays was excellent for detection of specific IgG and IgM antibodies, and discrepancies were relatively rare, i.e. in 3.6% of sera for IgG antibodies and 5.5% of sera for IgM antibodies. When results were compared between two assays (AxSYM *versus* VIDAS, and AxSYM *versus* VIDIA), the agreement was high for positive IgG results (99.6% and 99.6%, respectively), negative IgG results (98.8% and 99.0%, respectively), and negative IgM results (98.9% and 98.7%, respectively), and was lower for positive IgM results (81.5% and 92.9%, respectively).

Acute *T. gondii* infection in pregnant women may result in congenital diseases, causing abortion or severe damage to the fetus at birth or later in life. A specific and sensitive serological assay for toxoplasmosis is therefore crucial for the diagnosis of primary infection during pregnancy. To assess the specificity and the sensitivity of immunoassays, the choice of the reference method and of the tested samples must be considered carefully when comparing results published in different reports. In this study, sera were collected from pregnant women, and Sabin-Feldman dye test and ISAGA test were used to resolve discrepancies between AxSYM, VIDAS and VIDIA assays.

The performance of the three immunoassays was similar for the detection of IgG antibodies with high sensitivity (100.00% for VIDIA, 99.59% for VIDAS, 99.38% for AxSYM,) and specificity (99.04% for VIDIA, 98.82% for AxSYM, 98.57% for VIDAS). These results obtained with sera collected in Latin America are consistent with those reported for the same automated immunoassays with European sera, although no reference methods were used to determine the status of the sera.[19] A slightly higher sensitivity of the VIDIA IgG assay *versus* the VIDAS IgG was expected, as the VIDIA assay was calibrated to be more sensitive with a lower threshold of positivity than the VIDAS assay. Specificity and sensitivity of automated IgG immunoassays is usually high, and the proportion of false positive or false negative IgG results is low.[19,23-27] In this study, the few false negative IgG results obtained in VIDAS and AxSYM assays may be attributed to the high sensitivity of the dye test, which predominantly measures IgG antibodies, but also IgM and IgA antibodies, allowing both early and late diagnosis of *Toxoplasma* infection.[13,28] False positive IgG results were rare in this study and corresponded most often to low IgG antibody titers and to different sera with the three immunoassays. These false results may be due to the different nature of the antigen used in the ELISA and dye tests. In the dye test, membrane antigens present on the surface of live *T. gondii* are used for the detection of all Ig classes.[13,29] VIDAS IgG and AxSYM IgG assays use disrupted trophozoites (grown in mouse for the VIDAS assay and *in vitro* for AxSYM) exposing both membrane and cytoplasmic antigens. In the VIDIA IgG assay, the *T. gondii* antigen is produced *in vitro*, then immunopurified and corresponds mainly to a membrane antigen.

The specificity for the detection for IgM *T. gondii* antibodies was excellent for the three automated immunassays (99.88% for VIDIA, 99.76% for both AxSYM and VIDAS assays), showing only one false positive result for the VIDIA assay and two false positive results for both AxSYM and VIDAS assays. The specificity for the detection of *T. gondii* IgM antibodies has been questioned for some commercially available kits, and false positive IgM results have been reported.[30-33] The specificity of the ELISA IgM tests was then improved, using the immunocapture of IgM antibodies. As the interpretation of the presence of IgM antibodies is often complex, confirmatory tests, such as IgA ELISA, IgE ELISA, differential agglutination test and IgG avidity test are strongly recommended.[24,31,34,35] The IgG avidity test is widely used, and is based on the principle that antibody avidity gradually increases after exposure to an immunogen. Low avidity of IgG antibodies can be used as a marker for a recent primary infection. In this study, most of the false positive IgM

results were associated with low IgM titers. Two false positive IgM results (one for AxSYM and one for VIDIA) were true negatives for IgG antibodies, suggesting that the women had not been previously infected. Three false positive IgM results (one for AxSYM and two for VIDAS) were true positives for IgG, and two sera tested with VIDAS displayed a high avidity, suggesting a past infection. In the case of these three sera, it cannot be ruled out that VIDAS and AxSYM assays detected persisting IgM antibodies. As for IgG antibodies, the nature of the antigens used in ELISA IgM and the reference test is different. The ISAGA test incorporates entire, formalin fixed trophozoites and presents mainly membrane antigens.[29] In the AxSYM, VIDAS and VIDIA IgM assays, *T. gondii* antigens are whole-cell lysates, exposing both membrane and cytoplasmic antigens.

In contrast to the specificity, the sensitivity of the detection of IgM *T. gondii* antibodies differed between immunoassays. The VIDIA IgM assay displayed a higher sensitivity (95.12%) than VIDAS IgM (76.74%) and AxSYM IgM (61.90%) assays. In some subjects IgM may persist for many months or even years after acute infection.[36,37] Using the ISAGA test, IgM antibodies were shown to persist beyond 2 years in 27.1% of sera from *T. gondii*-infected pregnant women.[37] It has been reported that the ISAGA test can detect *T. gondii* IgM antibodies up to 12 years after primary infection, while other tests (ELISA, immunofluorescence, indirect hemagglutination) do not.[36] In our study, all false negative IgM results were positive for IgG antibodies with the three assays. For 13 of them, which could be tested with the VIDAS IgG avidity test, a high avidity was observed, suggesting that most of the false negative results were probably related to a past infection, which could still be detected by a highly sensitive test such as ISAGA.

Although the concordance was excellent for positive and negative results, the correlation between IgG titers was more limited between AxSYM and VIDAS assays, and between AxSYM and VIDIA assays. Antibody titers were approximately three times lower with the AxSYM assay, than with VIDAS and VIDIA assays. In contrast, antibody titers measured in VIDAS and VIDIA assays were very close. This may be related to the nature of the antigens used in the three tests, and the international standard used for calibration (WHO 1st for AxSYM, WHO 2nd for VIDAS, WHO 3rd for VIDIA). Anti-human IgG conjugates are also different in the AxSYM IgM assay (polyclonal serum) and VIDAS and VIDIA assays (monoclonal antibody). This inter-technique variability in IgG antibody titer was recently reported. Various sera were tested in six IgG automated immunoassays. The median of the results varied by a factor of 1 to 25 depending on the test used.[27] According to AFSSAPS (*Agence Francaise de Sécurité Sanitaire des Produits de Santé*) recommendations,[38] antibody titers or paired sera should only be compared using the same test.

In conclusion, our study performed with Latin American sera confirmed the excellent specificity of AxSYM, VIDAS and VIDIA assays for the detection of IgG and IgM *T. gondii* antibodies already reported in other countries. The sensitivity of the detection of IgG antibodies was slightly higher with VIDIA than with VIDAS and AxSYM assays. The sensitivity of the detection of IgM antibodies was higher with VIDIA than with VIDAS and AxSYM assays.

The determination of the immune status of pregnant women is essential for defining the appropriate follow-up and prophylactic measures. Therefore, positive or equivocal IgM results should be followed by confirmatory testing at a reference laboratory, and interpreted in association with IgG results and other tests, such as the IgG avidity test.[35]

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