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Comparative evaluation of the ARCHITECT Toxo IgG, IgM, and IgG Avidity assays for anti-*Toxoplasma* antibodies detection in pregnant women sera

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Abstract

We assessed the performance of the ARCHITECT Toxo IgG, IgM, and IgG Avidity assays against corresponding assays on AxSYM and Vidas using 730 sera from pregnant women. The ARCHITECT Toxo IgG and IgM assays showed a relative sensitivity of 97.5% and 89.9% and a relative specificity of 99.1% and 99.8%, respectively. If IgM sensitivity is calculated only for sera drawn less than 4 months after infection, the relative sensitivity rises to 98.1%. Correlation between the ARCHITECT and Vidas Avidity assays was 0.87 (n = 103). Testing 86 IgG-positive specimens from recent infection (<4 months), we never obtained high avidity results, but 2 specimens were in the gray zone, whereas sera from past infections (>4 months) exhibited high avidity results in 72.5% (137/189) of cases. The method can be used reliably to exclude recent infections in sera with concurrently positive results for IgM and IgG (IgG, >3 IU/mL).

Keywords: Toxoplasmosis; *Toxoplasma gondii*; Diagnosis; Serology; IgG, IgM; Avidity; Pregnant women

1. Introduction

When a *Toxoplasma gondii* primary infection is acquired during pregnancy, the parasite may be transmitted to the fetus. The overall maternal–fetal transmission rate has been estimated to be 29%, but the risk rises sharply with the duration of gestation, from 6% at 13 weeks to 72% at 36 weeks. The risk and the severity of clinical sequelae are also related to the time of the maternal infection, and the highest risk occurs at 24 to 30 weeks of gestation (Dunn et al., 1999). Primary infection is often asymptomatic in immunocompetent subjects, in which case, the initial diagnosis of *T. gondii* infection is mainly based on serologic methods. As the first serum is often drawn during the pregnancy, several approaches have been developed to determine whether the maternal infection has been acquired before or after conception. As a routine strategy, antibody assay combinations and comparison between first-line and second-line Toxo-IgG and Toxo-IgM tests have proven to be very useful. The first-line technique used as a screening test must be highly sensitive, whereas the second-line test must be both sensitive and specific. Moreover, if designed with different antigenic targets, the results of the 2 serologic tests can be compared to facilitate the differentiation of recent from chronic infection (Flori et al., 2008; Roberts et al., 2001). In combination with these conventional tests, the measurement of IgG avidity was developed almost 20 years ago to help for discrimination between recent and past infection (Hedman et al., 1989; Lappalainen and Hedman, 2004) based on the

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principle of the elution of low avidity antibodies by a protein-denaturing agent, mostly urea (Alvarado-Esquivel et al., 2002; Candolfi et al., 2007; Cozon et al., 1998; Fricker-Hidalgo et al., 2006; Petersen et al., 2005). For the ARCHITECT instrument, a new concept called AVIcomp has been developed to measure avidity. It is based on the removal of the high avidity fraction of marker-specific IgG by pretreatment of the specimen with soluble antigen before its addition to the solid phase (Curdt et al., 2009). The aim of the current study was to assess the performance of the complete panel of ARCHITECT Toxo assays, that is, Toxo IgG, Toxo IgM, and Toxo IgG Avidity using well-characterized pregnant women sera by other commercially available tests, namely, AxSYM IgG and IgM (Abbott Laboratories, Wiesbaden, Germany), Vidas IgG-II, IgM, and Avidity (bioMérieux, Marcy l'Etoile, France) with established performance (Calderaro et al., 2008; Hofgartner et al., 1997; Roux-Buisson et al., 2005; Wilson et al., 1997).

2. Materials and methods

2.1. Serum specimens

Seven hundred thirty samples from pregnant women were obtained from routine screening performed by the Parasitology–Mycology Laboratories of 2 French university hospitals: Grenoble (n = 546) and Nantes (n = 184). All samples included in the study had been assayed for IgG and IgM by 2 different commercial methods: microparticle immunoassay (MEIA) (AxSYM, Abbott Laboratories) and enzyme immunoassay (EIA) (Vidas, bioMérieux). The following titers were considered positive, negative, or equivocal, respectively, in the various tests: IgG AxSYM, ≥3.0, <2.0, 2.0 to 2.9 IU/mL; IgG Vidas, ≥8, <4, 4 to 7 IU/mL; IgM AxSYM, ≥0.600, <0.500, 0.500 to 0.599; IgM Vidas, ≥0.65, <0.55, 0.55 to 0.64. Resolution testing of specimens with discordant results between assays was done as follows: specimens with a positive IgG result in 1 assay and no IgM reactivity were assayed by an IgG in-house immunofluorescent assay (IFI) (Roberts et al., 2001) and the Sabin–Feldman dye test, to conclude the presence or absence of antibodies against *T. gondii*. Discordant specimens for IgM reactivity but no IgG antibodies were assayed with the IgM-immunosorbent agglutination assay (IgM-ISAGA) test (bioMérieux). Based on the resolved results and after biologic interpretation, samples were classified into 5 groups: group 1 (n = 401), IgG negative/IgM negative; group 2 (n = 79), IgG positive/IgM negative; group 3 (n = 12), IgG negative/IgM positive; group 4 (n = 164), IgG positive/IgM positive; and group 5 (n = 74), consisting of 28 seroconversion cases. In addition, the Vidas IgG Avidity assay was performed on some IgG-positive/IgM-positive specimens if the different results from above techniques were not sufficient to determine the date of the infection. Finally, according to clinical data (if available), previous results, comparison between first-line and second-line test results, and, when needed, Vidas avidity index, it was possible to divide group 4 into 2 groups: toxoplasmosis acquired in the preceding 4 months (n = 45) and toxoplasmosis older than 4 months (n = 119). All samples precharacterized by the methods outlined above were tested on the ARCHITECT Toxo IgG and Toxo IgM assays. The ARCHITECT Toxo IgG Avidity assay was used to test ARCHITECT Toxo IgG-positive sera (IgG, ≥3.0 IU/mL) or sera with gray zone results for IgG (1.6–2.9 IU/mL). Principles of the ARCHITECT assays are outlined below. Commercially available assays were performed according to package insert instructions.

2.2. ARCHITECT Analyzer

2.2.1. ARCHITECT Toxo IgG assay

The ARCHITECT Toxo IgG assay is a fully automated, 2-step chemiluminescent microparticle immunoassay (CMIA), designed for quantitative determination of IgG antibodies to *T. gondii*. *T. gondii*-specific antibodies present in the sample bind to the *T. gondii* recombinant antigen P30 (SAG1) and P35 (GRA8)-coated microparticles, forming an antigen–antibody complex (Sickinger et al., 2008). After washing, murine anti-human IgG acridinium-labeled conjugate is added in a second step to create a reaction mixture with *T. gondii*-specific IgG bound to the microparticles. After another wash cycle, pretrigger and trigger solutions are added to the reaction mixture. The final chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of Toxo IgG in the sample and the RLUs detected by the ARCHITECT optical system. Results are calculated automatically based on a previously established calibration curve. Specimens with concentration values ≥3.0 IU/mL are considered reactive for IgG antibodies to *T. gondii*, concentration values from 1.6 to 2.9 IU/mL are considered gray zone, and concentration values <1.6 IU/mL are considered nonreactive.

2.2.2. ARCHITECT Toxo IgM assay

The ARCHITECT Toxo IgM assay is based on μ-capture format in which the patient's IgM are bound to anti-human IgM mouse monoclonal antibody-coated paramagnetic microparticles. Specific detection of the anti-*Toxoplasma* IgM is accomplished via incubation with native *T. gondii* lysate, which is complexed to an acridinium-labeled anti-Toxo P30 mouse monoclonal F(ab′)2 fragment. During development, reactive results were defined as index values ≥0.35, gray zone results ranged from 0.29 to 0.34, and nonreactive results were defined as index values <0.29.

2.2.3. ARCHITECT Toxo Avidity assay

The ARCHITECT Toxo IgG Avidity assay is a CMIA for the determination of the avidity of IgG antibodies to *T. gondii* in human serum and plasma (Sickinger et al., 2008) using the novel AVIcomp methodology: The ARCHITECT Toxo IgG Avidity assay consists of 2 single tests, which are both 2-step immunoassays. The avidity of anti-Toxo IgG in the sample is calculated using RLUs of both tests. One

Table 1
Comparison of ARCHITECT results for IgG with the 2 commercial techniques (AxSYM and VIDAS) obtained on the group 1: IgG−/IgM− (n = 401)

Assay	Cutoff	No. of sample with the following ARCHITECT IgG result			
		Reactive	Gray zone	Nonreactive	Total
AxSYM IgG	Positive	0	0	0	0
	Equivocal	0	1	2	3
	Negative	2	3	393	398
	Total	2	4	395	401
Vidas IgG	Positive	0	0	0	0
	Equivocal	0	1	1	2
	Negative	2	3	394	399
	Total	2	4	395	401

aliquot of the sample is pretreated with a Toxo recombinant antigen containing blocking agent. A second aliquot of the sample is pretreated with buffer devoid of blocking agent. The percentage avidity is calculated from the RLUs obtained from the sample pretreated with the blocking agent and the RLUs obtained from the unblocked sample. The ARCHITECT software calculates the avidity as per the following formula: Avidity (%) = 100 × (1 − [blocked assay/unblocked assay]). The percentage avidity calculation allows specimen classification as low (<50.0% Avi), gray zone (50.0–59.9% Avi), or high (≥60% Avi).

2.3. Resolved relative sensitivity/specificity and agreement calculation

Relative sensitivity, relative specificity, and agreement calculation for the ARCHITECT Toxo IgG or IgM assay were calculated as follows:

Table 2
Comparison of ARCHITECT results for IgG and IgM with the 2 commercial techniques (AxSYM and VIDAS) obtained on the group 2: IgG+/IgM− (n = 79 for IgG and n = 73 for IgM)

Assay	Cutoff	No. of sample with the following ARCHITECT result			
		Reactive	Gray zone	Nonreactive	Total
AxSYM IgG	Positive	71	4	0	75
	Equivocal	0	2	0	2
	Negative	1	1	0	2
	Total	72	7	0	79
Vidas IgG	Positive	71	3	0	74
	Equivocal	1	4	0	5
	Negative	0	0	0	0
	Total	72	7	0	79
AxSYM IgM	Positive	0	0	0	0
	Equivocal	0	0	0	0
	Negative	1	1	71	73
	Total	1	1	71	73
Vidas IgM	Positive	0	0	0	0
	Equivocal	0	0	0	0
	Negative	1	1	71	73
	Total	1	1	71	73

Table 3
Comparison of ARCHITECT results for IgG with the 2 commercial techniques (AxSYM and VIDAS) obtained on the group 4: IgG+/IgM+ (n = 164)

Assay	Cutoff	No. of sample with the following ARCHITECT IgG result			
		Positive	Equivocal	Negative	Total
AxSYM IgG	Positive	161	2	1	164
	Equivocal	0	0	0	0
	Negative	0	0	0	0
	Total	161	2	1	164
Vidas IgG	Positive	157	0	0	157
	Equivocal	3	1	0	4
	Negative	1	1	1	3
	Total	161	2	1	164

- Relative sensitivity = (true positive/[true positive + false negative]) × 100%
- Relative specificity = (true negative/[true negative + false positive]) × 100%
- Agreement = (number of concordant samples/number of all tested samples on both assays) × 100%.

Results of relative specificity, relative sensitivity, and agreement are given with 95% confidence intervals. Specimens with gray zone results were excluded from relative specificity and relative sensitivity calculation, but detailed results including gray zone results are shown in different tables.

The χ^2 test was used to compare percentage of persistent IgM with 2 different assays; $P < 0.05$ was considered significant. The coefficient of correlation (r) was used to determine the statistical agreement between the 2 avidity assays.

3. Results

3.1. IgG and IgM analysis

3.1.1. Whole population

The agreement between ARCHITECT and AxSYM was 95.3% (696/730; confidence interval [CI], 92.9–97.0%) for Toxo IgG assays and 92.9% (659/709; CI, 90.0–95.0%) for Toxo-IgM assays. The agreement between ARCHITECT and Vidas was 93.8% (685/730; CI, 91.1–95.8%) for Toxo IgG assays and 94.4% (669/709; CI, 91.7–96.2%) for Toxo-IgM assays. The relative sensitivity and specificity of the ARCHITECT Toxo IgG assay were 97.5% (272/279; CI, 93.8–99.0%) and 99.1% (423/427; CI, 96.9–99.7%),

Table 4
Number of sera (%) with persistent IgM according to the technique used among the >4 months of infections from the group 4 (IgG+/IgM+)

	No. of sera (%), n = 119		
	Reactive	Gray zone	Nonreactive
ARCHITECT	85 (71.4)	16 (13.4)	18 (15.1)
AxSYM	100 (84.0)	8 (6.7)	11 (9.2)
Vidas	85 (71.4)	15 (12.6)	19 (16.0)

One hundred nineteen sera were analyzed with the 3 techniques.

Table 5
Detailed results of IgG, IgM, and Avidity index on the 28 seroconversion cases

	Date	Toxo IgG assays			Toxo IgM Index			ARCHITECT avidity		Vidas avidity	
		Archi IU/ml	Vidas IU/ml	AxSYM IU/ml	ARCHITECT	Vidas	AxSYM				
Case 1	6/9/2007	0.2	0	0	0.03	0.08	0.08	x		x	
	7/6/2007	0.5	0	1.1	1.32	1.74	0.82	x		x	
	8/28/2007	72	81	450.8	1.79	3.24	1.95	23.2%	low	0.056	low
Case 2	5/23/2007	4	0	1.5	4.39	4.81	4.56	5.5%	low	x	
	5/29/2007	5.7	1	3.3	4.49	4.83	4.74	11.7%	low	x	
Case 3	7/24/2007	1.8	2	2.2	0.89	1.61	0.84	17.2%	low	x	
	8/3/2007	8.8	13	12.7	0.76	1.42	0.78	29.5%	low	0.037	low
Case 4	8/18/2006	0.3	0	0	0.08	0.02	0.18	x		x	
	4/29/2007	39.2	79	156.4	0.68	1.96	1.01	24.1%	low	0.081	low
Case 5	1/25/2007	0.1	0	0	0.04	0.05	0.14	x		x	
	3/9/2007	7.2	16	50.2	4.25	4.16	3.84	0.1%	low	0.052	low
	3/21/2007	31.6	28	102.5	3.93	3.7	3.59	14.5%	low	0.046	low
Case 6	4/27/2007	1.6	0	0	0.08	0.1	0.08	78.6%	high	x	
	7/25/2007	148.1	187	633.2	2.15	4.84	1.88	30.8%	low	0.073	low
	8/30/2007	89.1	198	541.9	1.7	3.93	1.24	31.2%	low	0.099	low
Case 7	8/23/2007	0.3	0	0.5	2.91	4.1	4.52	x		x	
	8/31/2007	1.2	0	4.9	3.25	5.07	4.79	x		x	
Case 8	5/5/2006	9.2	0	1.1	0.58	1.58	1.147	5%	low	x	
	5/10/2006	12.9	0	3.8	0.62	1.6	1.157	3.6%	low	x	
Case 9	1/20/2007	1.3	1	4.4	1.55	2.27	2.41	x		x	
	1/26/2007	7.5	7	18.3	1.73	2.73	3.17	14.5%	low	x	
	2/5/2007	16.6	26	33.7	1.99	3.28	2.82	17.4%	low	0.02	low
Case 10	3/6/2007	0.5	0	1.2	1.22	1.88	2.21	x		x	
	5/2/2007	12.7	16	36.1	3.95	5.62	8.32	24.6	low	0.036	low
	8/30/2007	523.6	> 300	353.7	3.08	2.89	2.41	49.3	low	0.169	low
Case 11	7/26/2005	0.4	0	0.6	1.3	3.2	3.275	x		x	
	8/16/2005	2.2	1	5.7	nd	2.76	3.47	18.4%	low	x	
Case 12	1/18/2006	1	0	0.6	0.26	0.51	0.584	9,00%	low	x	
	1/31/2006	5.4	0	11.5	0.76	1.44	1.473	10.3	low	x	
Case 13	1/2/2007	0.7	0	0.1	0.02	0.05	0.17	x		x	
	2/2/2007	6.5	0	9.6	5.36	5.58	5.27	10.9%	low	x	
	2/6/2007	6.9	0	13.8	5.37	5.43	5.11	6.8%	low	x	
Case 14	5/14/2007	0.23	0	0	0.02	0.04	0.08	x		x	
	6/14/2007	9.2	3	9.9	3.37	4.07	3.8	21.7%	low	x	
Case 15	4/16/2007	0.1	0	0	0.03	0.06	0.06	x		x	
	5/11/2007	37.5	10	18.3	0.11	nd	nd	23.6%	low	0.036	low
	5/23/2007	27.3	34	77.9	3.61	5.77	2.35	19.4%	low	0.156	low
	8/21/2007	674.9	283	969.3	0.65	1.55	0.28	47.3	low	0.0178	low
Case 16	6/27/2005	3.1	1	3.2	0.58	1.83	1.209	24.6%	low	x	
	7/1/2005	7.4	3	8.5	0.74	2.14	1.323	18.5%	low	x	
Case 17	2/14/2007	0.2	nd	0	0.03	nd	0.14	x		x	
	3/19/2007	19.2	4	70.7	8.8	7.33	8.78	10%	low	x	
	4/2/2007	74.5	89	404.4	8.91	7.89	7.75	13.1%	low	0.051	low
Case 18	9/7/2005	0.4	0	2.1	0.56	2.05	1.93	x		x	
	9/10/2005	0.7	0	3	0.62	2.39	1.585	x	x	x	
Case 19	2/21/2007	0.2	0	0.4	0.04	0.08	0.12	x		x	
	3/14/2007	1	0	3.1	2.42	3.64	2.94	x	x	x	
	4/4/2007	3.3	2	14.1	2.66	3.66	3.32	13.1%	low	x	
Case 20	2/9/2007	2.2	0	1.2	0.77	2.8	2.25	58%	GZ	x	
	2/16/2007	3.9	2	4	0.9	2.93	2.32	39.6%	low	x	
	3/16/2007	38.7	60	70.1	0.79	1.97	1.73	41%	low	0.024	low
	9/9/2007	27.4	55	41.5	0.2	0.56	0.51	55.8%	GZ	0.135	low
Case 21	4/22/2005	0.3	0	1.2	1.77	2.76	3.242	x	x	x	
	5/9/2005	1.8	0	4.2	10.35	8.17	9.5	8.7%	low	x	
Case 22	5/30/2007	0.1	0	0	0.08	0.1	0.13	x	x	x	
	6/29/2007	1.5	0	3.3	nd	2.83	3.16	x	x	x	
	7/16/2007	17.9	12	29.2	2.51	2.89	3.55	22.1%	low	0.082	low
	7/26/2007	23.1	23	37.6	2.67	2.76	3.84	17%	low	0.58	low
Case 23	12/30/2005	2.4	0	6.4	0.88	1.58	2.582	12.3%	low	x	
	1/17/2006	5.8	2	10.5	0.47	1.01	1.693	6.3%	low	x	

Table 5 (*continued*)

	Date	Toxo IgG assays			Toxo IgM Index			ARCHITECT avidity		Vidas avidity	
		Archi IU/ml	Vidas IU/ml	AxSYM IU/ml	ARCHITECT	Vidas	AxSYM				
Case 24	6/27/2007	0.2	0	0.5	0.04	0.07	0.11	x	x	x	
	7/30/2007	0.3	0	0.8	0.9	0.9	1.28	x	x	x	
	8/20/2007	1	1	3.2	3.29	4.11	2.85	x	x	x	
	9/10/2007	14.5	58	126.4	7.77	8.91	6.76	37.4%	low	0.023	low
Case 25	7/31/2006	9.9	0	3.6	2.61	4.87	3.367	0.5%	low	x	
	8/18/2006	27.2	3	17.4	2.75	5.87	3.597	9.3%	low	x	
Case 26	1/4/2007	0.4	0	0	0.02	0.06	0.06	x		x	
	6/8/2007	111.6	210	261.2	1.6	3	1.25	24.3%	low	0.072	low
	6/30/2007	297	299	502.5	0.81	1.79	0.63	33.7%	low	0.079	low
Case 27	10/18/2007	1.9	0	1.5	2.53	3.6	3.91	6.7%	low	x	
	10/23/2007	3.7	0	3	3.43	4.24	4.7	8.3%	low	x	
	11/5/2007	7.8	2	11.5	0.91	5.02	4.95	18.9%	low	x	
Case 28	7/7/2007	10.7	1	2.5	2.22	3.4	1.47	3.5%	low	x	
	7/18/2007	48.3	10	44	5.25	7.8	3.58	5.7%	low	0.087	low

Detailed results of IgG, IgM, and Avidity index on the 28 seroconversion panels obtained with the 3 techniques: ARCHITECT, VIDAS, and AxSYM. Nonreactive results are not highlighted. Gray zone results are highlighted in light gray; reactive results are highlighted in dark gray. The measurement of avidity index was performed with ARCHITECT on IgG-positive and gray zone specimens (IgG, ≥1.6 IU/mL). The measurement of avidity index was performed with Vidas on positive samples only (IgG, ≥8 IU/mL), as prescribed by the manufacturer.

respectively. The relative sensitivity and specificity of the ARCHITECT Toxo IgM assay were 89.9% (195/217; CI, 83.3–94.0%) and 99.8% (466/467; CI, 98.2–100.0%), respectively. If IgM sensitivity is calculated only on samples from recent infections (<4 months), the sensitivity rises to 98.1% (105/107; CI, 93.4–99.5%).

3.1.2. Group 1 (IgG−/IgM−)

Table 1 shows a detailed comparison between results obtained by ARCHITECT and the 2 other commercial tests for IgG on 401 sera for which the biologic interpretation was the absence of antibodies against *T. gondii*. In this group, the relative specificity of ARCHITECT Toxo IgG was 99.5% (395/397). Seventeen specimens could not be tested for Architect Toxo IgM because of insufficient sample volume. All of the 384 samples assayed for IgM by the ARCHITECT system were nonreactive.

3.1.3. Group 2 (IgG+/IgM−)

All of the 79 sera evaluated for IgG by ARCHITECT were positive (n = 72) or within the gray zone (n = 7) (Table 2). In this group, 5 specimens exhibiting discordant results between AxSYM and Vidas were confirmed positive with Sabin–Feldman dye test. The 7 specimens with gray zone results for ARCHITECT also showed low IgG titers with other techniques (≤8 IU/mL for Vidas and ≤3.5 IU/mL for AxSYM), except 1 sera with IgG = 10.3 IU/mL for AxSYM. In this group, 73 specimens have been analyzed for IgM with ARCHITECT. Only 1 was reactive and 1 within the gray zone.

3.1.4. Group 3 (IgG−/IgM+)

Consisted of 12 specimens, which were negative for IgG with AxSYM and Vidas and positive or gray zone for IgM according to at least 1 of the routine tests used in our laboratories. Presence of IgM was confirmed by ISAGA for 7 specimens (ISAGA, ≥9). ISAGA was within the gray zone for 1 specimen and remained negative for 4 specimens. After resolution, based on the follow-up of the patients and/or IgM IFI, this group can be divided into 2 categories: nonspecific IgM (n = 9) and onset of seroconversion (n = 3). The 3 sera of the subgroup seroconversion were all IgM reactive with all of the 3 techniques. Nonspecific IgM were fewer with ARCHITECT than Vidas (7/9 versus 9/9) but more numerous than with AxSYM (7/9 versus 4/9).

3.1.5. Group 4 (IgG+/IgM+)

Consisted of 164 samples of patients with acquired infection positive for IgG and IgM with AxSYM and/or Vidas (Table 3). One sample was not reactive with ARCHITECT and 2 within the gray zone for IgG. The group 4 was then divided into 2 subgroups for IgM analysis: recent infections acquired within the preceding 4 months (n = 45) and infections acquired more than 4 months ago (n = 119). In the recent infection subgroup, IgM samples were positive in 100% (45/45) of assayed specimens with ARCHITECT and Vidas and 97.8% (44/45) with AxSYM. One IgM specimen showed a gray zone result with AxSYM (0.57). In the subgroup of infections acquired more than 4 months ago, persistence of IgM was studied in 119 specimens. Persistent IgM was less frequently detected by ARCHITECT (71.4%) than by AxSYM (84.0%) ($P < 0.05$) as shown in Table 4. Results were similar between ARCHITECT (71.4%) and Vidas (71.4%).

3.1.6. Group 5 (seroconversion cases)

Consisted of 28 seroconversion cases with 74 samples as shown in Table 5. In all cases except 1, IgM antibodies were detected concurrently by all the 3 methods. In case 12, IgM was gray zone reactive by AxSYM earlier than by other techniques. In 4 cases (2, 8, 20, and 27), IgG appeared first (gray zone or positive) by ARCHITECT, whereas IgG remained below the cutoff with AxSYM and was negative by Vidas (IgG = 0 IU/mL). In 10 cases (3, 11, 12, 13, 14, 16, 21,

Table 6
Avidity index results obtained with ARCHITECT Toxo IgG Avidity assay on 275 positive samples

	<4 months	>4 months	Total
High	0	137	137
Gray zone	2	31	33
Low	84	21	105
Total	86	189	275

The results were analyzed according to the time of seroconversion: <4 months of infections (n = 86) and >4 months of infections (n = 189).

23, 25, and 28) IgG was detected (gray zone or positive) concurrently by ARCHITECT and AxSYM, whereas IgG values for Vidas were below the cutoff. In another 6 cases (7, 9, 18, 19, 22, and 24), IgG was first detected by AxSYM, whereas IgG was negative with ARCHITECT (sometimes close to the gray zone) and with Vidas.

3.2. Toxo IgG Avidity assay

The ARCHITECT Toxo IgG Avidity assay has been performed on the 275 sera with positive results by ARCHITECT IgG (≥3.0 IU/mL), 86 of those were from recent infections (<4 months), the remaining 189 were from patients with past infection (>4 months) (Table 6). According to the package insert, ARCHITECT Toxo IgG Avidity assay may also be used for samples with low IgG titers and even gray zone results. In total, 22 such samples were tested. Of 8 past infections without IgM, 6 had a high avidity result, 1 exhibited a low avidity, and 1 specimen had a gray zone avidity result. Eight of 9 recent infection specimens (<4 months) had a low avidity result; only 1 specimen showed an avidity result within the gray zone, although it was the first serum of a seroconversion case (see case 20, Table 5). The last group (n = 5) consisted of sera for which IgG was not confirmed by other assays. The avidity index was low (n = 3) or in the gray zone (n = 1), but 1 sample showed a high avidity index despite being the first bleed of a seroconversion case 6 (Table 5).

The ARCHITECT Toxo Avidity assay was also compared with the results of the Vidas assay on the 103 IgG-positive sera, which have been analyzed with both techniques (19 <4 months; 84 >4 months). Results are shown in Fig. 1. The correlation coefficient was 0.87 (r^2 = 0.7518).

4. Discussion

The ARCHITECT Toxo IgG, IgM, and IgG Avidity assays have been developed as a fully automated panel for immune status determination and exclusion of acute infection. Some performance characteristics of the ARCHITECT Toxo IgG and Toxo IgG Avidity assays have been described earlier in comparison with the AxSYM Toxo IgG assay as well as the Vidas Toxo IgG Avidity assay (Sickinger et al., 2008), as well as the specificity and seroconversion sensitivity of the ARCHITECT Toxo IgM assay in comparison with the AxSYM Toxo IgM assay (Sickinger et al., 2009). The present study, however, is the first approach to evaluate the 3 new ARCHITECT Toxo assays (IgG, IgM, and Avidity) in comparison with results obtained in routine practice of reference laboratories for toxoplasmosis using several different techniques. Such comparison is of great importance to determine serodiagnostic strategies, which have to be based on the sensitivity and the specificity of different tests (Roberts et al., 2001; Sensini, 2006).

4.1. IgG and IgM assay analysis

4.1.1. Specificity

In the current study, the overall IgG specificity was calculated to be 99.1% (423/427; CI, 96.9–99.7%), resembling values of 99.6% specificity as obtained in a previous study (Sickinger et al., 2008). Among the discordant samples, 2 corresponded to onset of seroconversion (first serum from case 2 and case 8 from group 5) for which the IgG appeared earlier with ARCHITECT than with other assays and may be considered as true positive. The 2 other discordances correspond to 2 specimens from the group 1 (IgG−/IgM−) for which the positivity of ARCHITECT Toxo IgG was not confirmed by other techniques (AxSYM, Vidas). Both sera were also negative with IgG IFI (IgG, 0 IU/mL). One of these 2 specimens was also negative with Sabin–Feldman dye test, whereas the second one has not been analyzed with Sabin–Feldman dye test because of insufficient volume of sample. In group 1, 3 other specimens were within the gray zone with ARCHITECT and negative with all the other techniques (including IFI and Sabin–Feldman dye test). It has been shown that specificity (and sensitivity) depend on the antigens involved in the assay (Petersen et al., 2005; Roux-Buisson et al., 2005). The ARCHITECT Toxo IgG assay contains recombinant P30 (SAG1) and P35 (GRA8) antigens (Gatkowska et al., 2006; Lu et al., 2006). Complementary analysis with the recom-

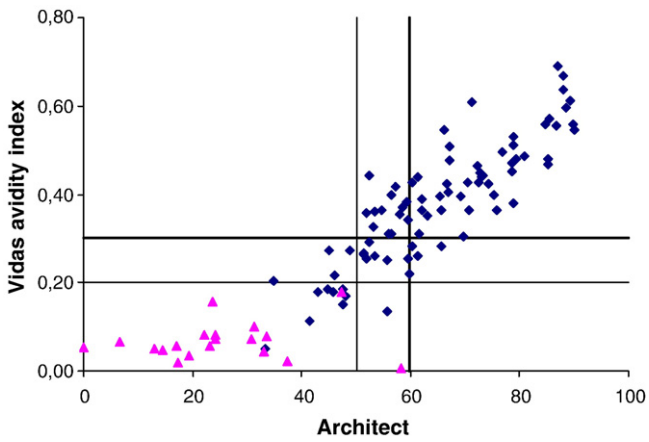

Fig. 1. Correlation of avidity index measured on 103 positive sera analyzed with both techniques (ARCHITECT and Vidas). Samples were classified according to the time of the infection: <4 month (▲) and >4 months (◆).

Line Toxo IgG Blot or other Western Blot techniques using P30 and P35 antigens has not been performed in the present study. Thus, we can only suspect a false positivity for the 2 positive specimens, but we know that in routine practice, very rare false-positive IgG results can be obtained with any test. Because of that, despite being a cost-intensive practice, many French parasitology laboratories support the systematic confirmation of IgG-positive results by a second-line test before concluding that the patient has immunity.

Concerning the IgM, a high specificity is very important because detection of IgM during pregnancy is highly distressing for the patient (Liesenfeld et al., 1997; Roberts et al., 2001). The risk of false-positive Toxo IgM results is well known and has been discussed in the context of many commercial tests. In our study, among all the negative specimens for IgM (corresponding to groups 1 and 2), only 2 sera, from the group 2, were discordant with the ARCHITECT system: 1 positive and 1 gray zone. The overall relative specificity was 99.8% (466/467; CI, 98.2–100.0%). These discordant results were due to low persistent IgM detected by ARCHITECT (0.43 and 0.3), whereas the corresponding IgM titers with AxSYM and Vidas were just below the cutoff. On the contrary, we observed that these persistent IgM profiles are less frequently detected with ARCHITECT than with AxSYM. This observation is important for routine practice because IgG-positive/IgM-negative samples do require less confirmatory testing than IgG-positive/IgM-positive samples. Nevertheless, detection of IgM around the cutoff in a patient with positive IgG is not really a critical situation. Usually, it is easy to rule out recent infection by a second-line IgM assay in a combinatorial strategy (Roberts et al., 2001). If needed, the measure of the avidity index will help to exclude a recent infection, and the biologic interpretation will be confirmed on a second sample drawn some weeks later (Pelloux et al., 2006).

4.1.2. Sensitivity

If the sensitivity of a Toxo IgG test is insufficient, too many women will be enrolled in an unnecessary follow-up (which is cost intensive for the health system and inconvenient for the pregnant women). The analysis of the group 2 suggests a slightly lower sensitivity of ARCHITECT IgG compared with AxSYM, which is known for very high sensitivity, especially for low IgG titer specimens (Cimon et al., 1998; Roux-Buisson et al., 2005). Among the 7 gray zone specimens with ARCHITECT, 3 were positive with AxSYM and Vidas. Two were also gray zone with AxSYM and Vidas (Sabin–Feldman dye test positive), and 2 were gray zone with Vidas (1 positive and 1 negative with AxSYM). Sabin–Feldman dye test was positive on these last 4 discordant specimens. For all of the 7 gray zone specimens, IgM was negative with all the techniques, and we assume that these specimens correspond to past infections.

The sensitivity of IgG and IgM is also crucial for the precocity of the seroconversion diagnosis. The analysis of 28 seroconversion cases shows equivalent IgM performance for the 3 tests. Concerning the Toxo IgG kinetics, we confirmed the greater sensitivity of ARCHITECT and AxSYM IgG versus Vidas. In general, the ARCHITECT and AxSYM seroconversion results are equivalent and depend on the case studied. This sensitivity in the detection of the IgG is very helpful in routine practice to confirm a toxoplasmic infection suspected by rising IgM levels. This is essential for the management of toxoplasmosis because the treatment, although still controversial, could be more efficacious when administered early after seroconversion (Thiebaut et al., 2007).

4.2. Toxo IgG Avidity analysis

The ARCHITECT Toxo IgG Avidity assay is a qualitative method for the determination of the avidity of IgG antibodies to *T. gondii* in human serum or plasma. It is proposed as an aid in the differentiation between recent and past *T. gondii* infection.

The present study showed that a high avidity index with the ARCHITECT assay, associated with IgG and IgM seroreactivity, is a good indicator that an acute *T. gondii* infection within the last 4 months can be excluded. Moreover, among 189 past infections (>4 months) investigated in this study, the measure of avidity would have allowed to take accurate conclusions in 72.5% of cases. The other cases correspond to a slow maturation of avidity, a phenomenon that has already been described for several avidity assays. These studies have shown that low avidity results may persist for several years depending on the individual (Fricker-Hidalgo et al., 2006; Lefevre-Pettazzoni et al., 2006; Remington et al., 2004). Therefore, a low avidity index should not be considered as a sufficient argument to confirm a recent seroconversion and even less to prescribe invasive procedures like amniocentesis. Furthermore, the comparison of results from different studies must be carefully considered because the factors, which influence the maturation of avidity, are not well understood. The avidity values and interpretations may depend on the different techniques used to measure avidity. However, in our study, the comparison between the 2 commercial tests, ARCHITECT and Vidas, has shown a high correlation coefficient (r = 0.87) for these qualitative test methods, despite different concepts. Previous comparisons of avidity assays have found correlations of about 80% (Alvarado-Esquivel et al., 2002; Petersen et al., 2005), but in other studies, significant discrepancies have been observed (Lefevre-Pettazzoni et al., 2006). It is also hypothesized that treatment prescribed to prevent transplacental transmission could slow down the maturation of avidity (Candolfi et al., 2007; Lefevre-Pettazzoni et al., 2007). In our study, this parameter has not been taken into account. Another essential point to be considered for any avidity assay is that, in cases of recent toxoplasmosis infection, very exceptionally, nonexpected results (high avidity) can be obtained (Fricker-Hidalgo et al., 2006; Jenum et al., 1997; Martin

and Morin, 2006; Petersen et al., 2005). In our study, among the IgG-positive specimens analyzed for avidity, only 1 recent sample has been found within the gray zone (58.2%). However, it is worth noting that, for the same patient, another serum drawn 2 weeks later has shown a low avidity index (35.8%). Such a discordant kinetic profile is rare but has already been described (Fricker-Hidalgo et al., 2006). Another similar case has been observed in our study but for a specimen with a low IgG titer, within the gray zone (first serum of case 20, Table 5). Finally, 3 other discordant results have been observed, all 3 related to specimens with low titers of IgG in the absence of IgM, which therefore would not have been tested for IgG avidity per normal laboratory procedures (first serum of case 6 and 2 samples from patients that were considered nonimmune). For these samples, the IgG positivity, and thus their specificity, could not be confirmed.

In conclusion, this study shows that the ARCHITECT is a valuable system for the analysis of all clinical situations concerning *T. gondii* serology. The high sensitivity of the ARCHITECT Toxo IgG assay, which is only slightly lower compared with AxSYM, combined with the seroconversion sensitivity and the specificity of the IgM assay make it a suitable tool to detect recent infection. The avidity assay has been demonstrated to have the utility to exclude recent infection. The results demonstrate that the novel methodology (AVIcomp) used to measure avidity of Toxo IgG antibodies on the ARCHITECT system provides results that are well comparable with the standard urea-based test methods. Although the ARCHITECT Toxo IgG avidity assay has been shown to accurately determine the avidity of many gray zone samples, we recommend to use it only on Toxo-positive IgG samples (IgG, >3 IU/mL) in conjunction with the Toxo IgM results. Moreover, a confirmation with a second sample drawn at least 3 weeks after the first one remains essential for a correct medical decision.

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