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Performance of the BioPlex 2200 Flow Immunoassay in Critical Cases of Serodiagnosis of Toxoplasmosis

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The BioPlex 2200 automated analyzer (Bio-Rad Laboratories, Hercules, CA) is a recently developed multiplex analyzer that enables the detection of anti-*Toxoplasma*, -rubella, and -cytomegalovirus antibodies in the same assay. The aim of this study was to compare this new technology (using the BioPlex 2200 ToRC IgG/IgM kit) in critical cases of serodiagnosis of toxoplasmosis (acute, chronic, or congenital infections and cases with discrepant results) to the technologies used in our routine practice, i.e., the Platelia IgG/IgM enzyme-linked immunosorbent assays (ELISAs) (Bio-Rad Laboratories) and the Toxo-Screen direct agglutination assay (bioMérieux, Lyon, France). Overall, most cases of false-positive/negative results obtained with the Platelia IgG or Toxo-Screen assay were corrected by the BioPlex 2200 ToRC IgG (87.5%). Furthermore, the analysis of 35 sequences of sera showed a trend toward a more rapid decrease of IgM titers by BioPlex 2200 than by Platelia. These results for IgM detection can be explained by a weaker detection of residual IgM. Indeed, among 23 serum samples from patients with probable past infection with long-lasting IgM (Platelia M positive and IgG avidity index, ≥0.5), the BioPlex 2200 *Toxoplasma* IgM assay was positive for only 11 serum samples. In our panel of critical cases comprising 156 serum and 6 cord blood samples from 103 patients with acute, chronic, or congenital infection, the BioPlex 2200 IgG assay was a sensitive (97.8%) and specific (91.3%) method for IgG detection. The high specificity (97.4%) of IgM detection combined with the shorter kinetics of IgM titers may considerably reduce the number of residual IgM detections, thus yielding more precise diagnoses of acute infections.

Infections due to the protozoan parasite *Toxoplasma gondii* are highly prevalent among humans and animals worldwide and are responsible for severe complications in immunocompromised patients and in children born to infected mothers. Diagnosis of this infection is therefore important not only for treatment but also for epidemiology and prevention (1). The use of serologic tests to search for anti-*T. gondii* antibodies is a primary method of diagnosis (2). Different commercial serologic tests are available and possess unique patterns of increases and decreases with time after infection (3–5). The tests most commonly used in routine laboratories for the detection of anti-*T. gondii* IgG are the double-sandwich enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent-antibody assay (IFA), the indirect hemagglutination assay, and the direct agglutination test. For anti-*T. gondii* IgM, the most common tests are the double-sandwich ELISA, the immunosorbent agglutination assay (ISAGA), and the IFA. Nevertheless, these techniques have certain disadvantages, such as significant hands-on time or low throughput (6).

The recently developed BioPlex 2200 automated analyzer (Bio-Rad Laboratories) is a multiplex flow immunoassay (MFI) that enables the simultaneous detection and identification of multiple antibodies in a single sample (7, 8). The ToRC IgG/IgM tests used in this study are two-step immunoassays that can detect anti-*Toxoplasma*, -rubella, and -cytomegalovirus antibodies simultaneously. Antigen-coated fluoromagnetic beads are used as the solid phase for immobilizing the related antibodies. After wash steps, a fluorescent reporter conjugate is added to probe for specifically bound analytes. The bead mixture is then passed through the detector; the first laser identifies each bead based on the specific dye coated on its surface, and the second laser determines the amount of antibody bound based on the fluorescence of the conjugate. This assay seems to be more sensitive than traditional immunoassays, has a high-throughput capacity, and provides a wide analytical dynamic range (9).

As *T. gondii* can cause severe congenital infection and can be responsible for life-threatening reactivations in immunocompromised patients, serological screening and diagnosis of toxoplasmosis require robust and specific IgG and IgM assays. The BioPlex 2200 ToRC IgG and IgM immunoassays already met these criteria in a prospective study on 600 serum samples subjected to routine testing (6). In another study, elevated sensitivity of IgM antibodies produced by recent infections was demonstrated (10). To complement these studies, we examined sera that were selected for their critical relevance for diagnosis or screening in different clinical contexts of toxoplasmosis.

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MATERIALS AND METHODS

Study design. A total of 156 serum and 6 cord blood samples (from 103 patients with acute, chronic, or congenital infection) selected over 5 years from our routine practice of *Toxoplasma* serology testing by Platelia IgG/IgM (Bio-Rad Laboratories, Marnes-la-Coquette, France) and Toxo-Screen direct agglutination (DA) IgG (bioMérieux, Lyon, France) were tested in a blind manner by the BioPlex 2200 ToRC IgG and IgM immunoassays using the BioPlex 2200 automated analyzer (Bio-Rad). The different situations (acute, chronic, and congenital infections) were categorized according to biological results and clinical diagnosis. Each serum

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TABLE 1 Categories of sera and assay results[a]

Category (*n*)	Results for:				
	Platelia IgG	Platelia IgM	Toxo-Screen	IgG avidity index	Toxo II IgG
Recent infection (43)	Positive	Positive	Positive	<0.5	
Apparent long-lasting IgM (23)	Positive	Positive	Positive	>0.5	
Platelia false-negative results (2)	Negative	Negative	Positive		Positive
Toxo-Screen false-negative results (3)	Positive	Negative	Negative		Positive
Platelia false-positive results (9)	Positive	Negative	Negative		Negative
Toxo-Screen false-positive results (2)	Negative	Negative	Positive		Negative

[a] $n = 162$. Sequential sera from patients with acute infections ($n = 80$) are not shown because patients had several serum samples with different results.

sample met one of the following criteria: (i) evidence of a recent infection ($n = 43$ serum samples, 37 with IgG/IgM-positive results and a low avidity index [AI] [<0.4] and 6 with IgG/IgM-positive results and an intermediate avidity index [between 0.4 and 0.5]), (ii) apparent long-lasting IgM ($n = 23$ serum samples with IgG/IgM-positive results and a high avidity index [>0.5]), (iii) discrepant IgG results between the Platelia IgG test and the Toxo-Screen DA IgG assay ($n = 16$ samples), or (iv) sequential serum samples of acute infections ($n = 80$ samples collected from 35 patients). Samples showing discrepant results after the initial testing were arbitrated by two confirmatory tests, the Toxo II IgG confirmation test for IgG (LDBIO Diagnostics) (11, 12) and the immunosorbent agglutination assay (ISAGA) for IgM (bioMérieux), which allowed the identification of 11 serum samples with false-positive results for IgG (9 with Platelia and 2 with Toxo-Screen) and 5 samples with false-negative results for IgG (2 with Platelia and 3 with Toxo-Screen) in our routine practice. In addition, an IgG avidity index was determined in sera with anti-*T. gondii* IgM and IgG.

Enzyme-linked immunosorbent assay and direct agglutination assay. Routine anti-*T. gondii* IgG and IgM screening tests were carried out by the quantitative Platelia IgG/IgM ELISAs (Bio-Rad) and by the Toxo-Screen DA IgG test (bioMérieux) as a second routine test for IgG. The test results were interpreted as negative, equivocal, or positive based on defined thresholds outlined in the manufacturer's instructions.

IgG avidity index. The avidities of anti-*Toxoplasma* IgG antibodies were determined by using the Platelia Toxo IgG avidity test (Bio-Rad). Based on the manufacturer's instructions, the avidity indexes were classified as follows: <0.4, low avidity; 0.4 to <0.5, intermediate avidity; and ≥0.5, high avidity (high avidity suggests an infection >20 weeks previously) (13).

Multiplex flow immunoassay. In addition to routine testing by ELISA/DA, each specimen was tested according to the manufacturer's instructions using the BioPlex 2200 ToRC IgG and IgM kits on the BioPlex 2200 analyzer. The principle of multiplex flow immunoassay technology has been reviewed previously (14, 15). For the anti-*T. gondii* IgG expressed in IU/ml, the thresholds used as interpretive criteria were established by the manufacturer, and the results were defined as negative (titer < 10), equivocal ($10 \leq$ titer < 12), or positive (titer ≥ 12). For the anti-*T. gondii* IgM expressed in the index, the thresholds used as interpretive criteria were established by the manufacturer, and the results were defined as negative (index < 0.9), equivocal ($0.9 \leq$ index < 1.1), or positive (index ≥ 1.1).

Statistical analyses. Statistical analyses were performed using the VassarStats website (Vassar College, NY). Agreement among the tests was measured by calculating the kappa (κ) coefficient (16) and was categorized as almost perfect ($\kappa > 0.8$), substantial ($0.8 > \kappa > 0.6$), moderate ($0.6 > \kappa > 0.4$), fair ($0.4 > \kappa > 0.2$), slight ($0.2 > \kappa > 0.01$), or poor ($\kappa < 0.01$). Equivocal BioPlex 2200 results were considered positive for sensitivity and specificity calculations.

RESULTS

General comparison between routine practice and BioPlex 2200 ToRC IgG/IgM assays in 162 sera. We investigated different clinical contexts of toxoplasmosis (acute, chronic, and congenital infections) and tested the corresponding serum samples with the BioPlex 2200 ToRC IgG and IgM tests. On this particular panel of sera (Table 1), the BioPlex 2200 *Toxoplasma* IgG assay showed high sensitivity and specificity (97.8% and 91.3%, respectively) (Table 2). The κ coefficient of 0.87 suggested almost perfect agreement between the BioPlex 2200 and our routine practice for the IgG assay. The BioPlex 2200 *Toxoplasma* IgG assay was more sensitive and specific than the Platelia IgG assay but less sensitive than the Toxo-Screen and Toxo II IgG confirmation test in acute infection (data not shown). The BioPlex 2200 *Toxoplasma* IgM assay showed good sensitivity and high specificity (85.5% and 97.4%, respectively) (Table 2). Excluding serum samples with residual IgM, the BioPlex 2200 *Toxoplasma* IgM assay showed better sensitivity and specificity (95.5% and 97.3%, respectively). However, the BioPlex 2200 *Toxoplasma* IgM assay seemed to be less sensitive in cord blood (2 out of 3 positive cord blood samples were not detected by the BioPlex 2200 IgM test) than the Platelia IgM assay. The κ coefficient of 0.72 suggested substantial agreement between the BioPlex 2200 and our routine practice, an IgM assay.

Comparison between ELISA IgM and the BioPlex 2200 ToRC IgM assay in 67 serum samples with low/intermediate or high IgG avidity index. Among 43 serum samples from presumed recent infections (Platelia IgM positive and an IgG avidity index of <0.49), the BioPlex 2200 *Toxoplasma* IgM assay showed an agreement of 95.3% (41/43) with the Platelia IgM assay (Table 3). To discriminate between false-positive and false-negative results in the two remaining serum samples, an ISAGA was performed. In the first sample, the ISAGA score was 7, suggesting a past infection with persisting low avidity. In the second sample, the ISAGA score was 12, suggesting either a false-negative result by the BioPlex 2200 ToRC IgM assay or shorter kinetics of an IgM decrease. Among 23 serum samples from patients with a probable past infection with long-lasting IgM (Platelia M positive and an IgG avidity index of ≥0.5), the BioPlex 2200 *Toxoplasma* IgM assay was positive in 11 (47.8%).

Comparison between ELISA/Toxo-Screen IgG discrepant results and the BioPlex 2200 ToRC IgG assay in 16 serum samples. Among 9 cases of false-positive Platelia IgG results, BioPlex 2200 ToRC IgG was positive in only 2 (Table 4). None of the 2 serum samples that gave a false-positive Toxo-Screen result were positive by BioPlex 2200. In 2 cases of false-negative Platelia IgG results and 3 cases of false-negative Toxo-Screen results, the BioPlex 2200 IgG was positive. Overall, 87.5% (14/16) of the false-positive/negative results obtained with Platelia IgG or Toxo-Screen were corrected by BioPlex 2200 ToRC IgG for toxoplasmosis.

TABLE 2 Comparison of the BioPlex 2200 IgG/IgM anti-*Toxoplasma* assays to routine practice using serum specimens from different clinical contexts[a]

Assay type and results	No. of specimens tested by routine practice[b]		Sensitivity (% [95% CI])[c]	Specificity (% [95% CI])	Agreement (% [95% CI])	κ coefficient (95% CI)	PPV[d] (% [95% CI])	NPV[e] (% [95% CI])
	Positive	Negative						
BioPlex 2200 *Toxoplasma* IgG								
Positive	136	2[f]	97.8 (93.3–99.4)	91.3 (70.5–98.5)	96.9 (92.6–98.9)	0.87 (0.77–0.98)	98.5 (94.3–99.7)	87.5 (66.5–96.7)
Negative	3[g]	21						
BioPlex 2200 *Toxoplasma* IgM								
Positive	106	1[f]	85.5 (77.7–90.9)	97.4 (84.6–99.8)	88.3 (82.1–92.6)	0.72 (0.60–0.83)	99.1 (94.1–99.9)	67.3 (53.2–78.9)
Negative	18[h]	37						

[a] $n = 162$.
[b] If Platelia IgG and Toxo-Screen DA had discordant results, the Toxo II IgG confirmation test was performed. Immunosorbent agglutination assays (IgM) were performed in critical cases. Equivocal results of the BioPlex 2200 assay were considered positive results in statistical calculations.
[c] CI, confidence interval.
[d] PPV, positive predictive value.
[e] NPV, negative predictive value.
[f] BioPlex 2200 false-positive result.
[g] Samples were negative by the Platelia IgG but positive or equivocal by the Toxo-Screen DA and positive by the Toxo II IgG confirmation tests. The same serum samples showed IgM-positive results by the Platelia IgM test, meaning very acute infections.
[h] Among these serum samples, 13 had a high IgG avidity index with long-lasting IgM, 2 cord blood samples were positive by Western blot IgM and/or Platelia IgM, 2 serum samples had apparent long-lasting IgM with a low IgG avidity index, and 1 serum sample was positive by Platelia IgM, corresponding to highly recent acute infection.

Analysis of sequences of sera with BioPlex 2200 ToRC IgG/IgM assays. An analysis of 35 sequences of sera collected from patients with acute or subacute infection showed a trend toward a more rapid decrease of IgM titers by BioPlex 2200 than by Platelia, as shown in Table 5. In one case of proven seroconversion with no IgM antibodies detected by Platelia, BioPlex 2200 detected IgM antibodies, confirmed by an ISAGA (Table 5, patient 3). The kinetics of IgM appearance were similar to that of the Platelia IgM assay in other serum samples (patients 4, 5, 6, 7, and 8). The concordance was total between the BioPlex 2200 ToRC IgG/IgM and Platelia IgG/IgM ELISAs for the samples from the 27 other patients.

DISCUSSION

Toxoplasma gondii is estimated to infect one-third of the world's human population and can be responsible for severe complications in pregnant women and immunocompromised patients, particularly individuals with HIV infection. Serological testing plays a crucial role in the screening and follow-up of the population (17–19), because this technique is relatively prompt and inexpensive and can usually define the different phases of infection (i.e., acute or chronic) or exclude a previous infection. The main drawbacks of serological testing are low throughput and long hands-on time for the manual assay. In many countries, the requirement for high-quality processes in medical laboratories favors the use of fully automated assays in order to respond to specific quality criteria. The recently developed BioPlex 2200 automated analyzer corresponds to these criteria. This analyzer can detect different antibodies in a single tube, thus yielding high-throughput multiplexing analysis, saving time and preventing errors of result transmission. To our knowledge, only one study assessed the ToRC IgG and IgM immunoassays for toxoplasmosis serodiagnosis (6). In that study, the BioPlex 2200 ToRC IgG assays demonstrated a high agreement of 98.7% (592/600 specimens) with the routine practice used by the authors, while the ToRC IgM assays yielded an agreement of 91.2% (547/600 specimens). However, these results were limited by the lack of clinical information and the low number of IgM-positive samples. Our study completes these results by assessing different sera selected for their critical relevance for the diagnosis or screening of toxoplasmosis

TABLE 3 Comparison of the BioPlex 2200 IgM anti-*Toxoplasma* assay to routine testing with Platelia IgM using serum specimens with low/intermediate or high avidity index

IgG avidity index[a]	No. IgM positive by Platelia	No. IgM positive by BioPlex 2200	Agreement (%)
<0.49	43	41[b]	95.3
≥0.5	23	11	47.8

[a] $n = 67$ serum samples from 67 patients.
[b] In the two remaining serum samples, the ISAGA scores were 7 and 12, with IgG avidity indexes of 0.27 and 0.21, respectively.

TABLE 4 Comparison of BioPlex 2200 IgG anti-*Toxoplasma* assay to routine testing with Platelia IgG/Toxo-Screen IgG using serum specimens with false-positive or false-negative results by the Platelia IgG or direct agglutination assay[a]

Assay type and result (*n*)	No. BioPlex 2200 IgG positive	No. BioPlex 2200 IgG negative	Correction (%)
Platelia IgG			
False positive (9)	2	7	77.8
False negative (2)	2	0	100
Toxo-Screen			
False positive (2)	0	2	100
False negative (3)	3	0	100

[a] $n = 16$ serum samples from 16 patients. All samples were confirmed by the Toxo II IgG confirmation test.

TABLE 5 Analysis of sequences of sera with BioPlex 2200 ToRC IgG/IgM assays

Patient	Date (mo/day/yr)	Platelia IgG[a]	Platelia IgM[b]	Toxo-Screen	IgG avidity index[c]	BioPlex 2200 IgG[d]	BioPlex 2200 IgM[e]
1	07/03/2008	182	19	Positive	0.08	250	4.0
	07/17/2008	229	23	Positive		420	4.0
	02/05/2009	2,400	5	Positive		900	0.8
2	12/14/2006	19	15	Equivocal		24	3.6
	02/09/2007	194	12	Positive		356	0.5
3	09/25/2007	0	0	Negative		3	1.5
	10/17/2007	0	0	Negative		6	1.3
4	12/09/2009	0	0	Negative		3	0.2
	01/12/2010	27	27	Positive	0.16	57	4
5	08/29/2007	0	0	Negative		3	0.2
	10/03/2007	3	21	Equivocal		9	4
6	11/29/2007	0	0	Negative		3	0.2
	02/08/2008	15	16	Positive	0.28	21	4
7	09/27/2007	0	0	Negative		3	0.2
	12/03/2007	10	18	Equivocal		51	4
8	12/23/2009	0	0	Negative		3	0.2
	01/28/2010	0	2	Equivocal		6	1.5

[a] Cutoff values for Platelia IgG: negative (titer < 6), equivocal ($6 \leq$ titer < 9), and positive (titer ≥ 9).
[b] Cutoff values for Platelia IgM: negative (index < 0.8), equivocal ($0.8 \leq$ index < 1), and positive (index ≥ 1).
[c] Cutoff values for Platelia avidity of IgG: negative (<0.4); equivocal ($0.4 \leq$ avidity < 0.5), and positive (index ≥ 0.5).
[d] Cutoff values for BioPlex IgG: negative (titer < 10), equivocal ($10 \leq$ titer < 12), and positive (titer ≥ 12).
[e] Cutoff values for BioPlex IgM: negative (index < 0.9), equivocal ($0.9 \leq$ index < 1.1), and positive (index ≥ 1.1).

(discrepant results with Platelia and Toxo-Screen, acute and chronic infections and congenital infections).

In our panel of critical cases, the BioPlex 2200 ToRC IgG assay showed a high positive predictive value (136/138, 98.6%) and a good negative predictive value (21/24, 87.5%). These results are concordant with those of a previous study (6), although we studied a very special panel of sera that were either collected from patients with acute, subacute, or congenital infection or that yielded inconsistent or discrepant results by conventional serological methods. The peculiarity of this panel highlights some major benefits but also shows a few limitations of the BioPlex 2200 assays for diagnosis. In our study, the three false-negative results by both BioPlex 2200 IgG and Platelia IgG were found in three recent acute-infection cases with the presence of anti-*Toxoplasma* IgM (detected by the Platelia IgM assay and the BioPlex 2200 ToRC IgM assay). In these cases, IgG seroconversion was reported by BioPlex 2200 3 weeks later. The IgG detection by the BioPlex 2200 ToRC IgG assay seems to be less sensitive in very acute infections than the Toxo-Screen DA test and Toxo II IgG confirmation test. Conversely, the BioPlex 2200 ToRC IgG assay corrected most of the false-negative and false-positive results obtained with the Platelia IgG assay and the Toxo-Screen DA test (14/16), preventing the use of expensive confirmatory tests. In light of these results, the fully automated BioPlex 2200 analyzer using the BioPlex 2200 ToRC IgG assay can replace our double-determination tests for anti-*Toxoplasma* IgG screening.

The results obtained with the BioPlex 2200 ToRC IgM assay are also in agreement with those of a previous study (10). Overall, the BioPlex 2200 ToRC IgM assay has been shown to be a highly specific and sensitive method for acute infections (IgG avidity index, <0.5) and often does not detect residual IgM (IgG avidity index, ≥ 0.5). We found a trend toward an earlier decrease of IgM antibodies after infection using the BioPlex 2200 ToRC IgM assay than that with the Platelia IgM test and the ISAGA score. If these particular kinetics were confirmed in a larger number of serum samples, they may be advantageous in the follow-up of infection but may also be problematic in interpreting the first screening of pregnant women when performed during the third trimester. Indeed, a less sensitive detection of residual IgM at the third trimester could impair the detection of acute infections occurring during the first trimester. Based on the sequences performed, the kinetics of the IgM titers by the BioPlex 2200 IgM assay might decrease from the 4th or 5th month. Concerning pregnant women in their second or third trimester without prior serological results, another test should be used to detect IgM in order to avoid this problem. A multicentric prospective study is warranted to refine our results using a larger number of samples.

In conclusion, the BioPlex 2200 IgG assay is a sensitive and specific method for IgG detection that can replace two techniques. Additionally, the high specificity of IgM detection by the BioPlex 2200 IgM assay combined with the shorter kinetics of IgM titers may considerably reduce the number of residual IgM detections, thus preventing the use of expensive and time-consuming confirmation techniques for more precise diagnosis of acute infection.

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REFERENCES

1. **Fleck DG.** 1989. Annotation: diagnosis of toxoplasmosis. J. Clin. Pathol. **42:**191–193. http://dx.doi.org/10.1136/jcp.42.2.191.
2. **Montoya JG.** 2002. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J. Infect. Dis. 185(Suppl 1)**:**S73–S82. http://dx.doi.org/10.1086/338827.
3. **Maudry A, Chene G, Chatelain R, Patural H, Bellete B, Tisseur B, Hafid J, Raberin H, Beretta S, Sung RT, Belot G, Flori P.** 2009. Bicentric evaluation of six anti-*Toxoplasma* immunoglobulin G (IgG) automated immunoassays and comparison to the Toxo II IgG Western blot. Clin. Vaccine Immunol. **16:**1322–1326. http://dx.doi.org/10.1128/CVI.00128-09.
4. **Villard O, Cimon B, Franck J, Fricker-Hidalgo H, Godineau N, Houze S, Paris L, Pelloux H, Villena I, Candolfi E.** 2012. Evaluation of the usefulness of six commercial agglutination assays for serologic diagnosis of toxoplasmosis. Diagn. Microbiol. Infect. Dis. **73:**231–235. http://dx.doi.org/10.1016/j.diagmicrobio.2012.03.014.
5. **Gay-Andrieu F, Fricker-Hidalgo H, Sickinger E, Espern A, Brenier-Pinchart MP, Braun HB, Pelloux H.** 2009. Comparative evaluation of the ARCHITECT Toxo IgG, IgM, and IgG Avidity assays for anti-*Toxoplasma* antibodies detection in pregnant women sera. Diagn. Microbiol. Infect. Dis. **65:**279–287. http://dx.doi.org/10.1016/j.diagmicrobio.2009.07.013.
6. **Binnicker MJ, Jespersen DJ, Harring JA.** 2010. Multiplex detection of IgM and IgG class antibodies to *Toxoplasma gondii*, rubella virus, and cytomegalovirus using a novel multiplex flow immunoassay. Clin. Vaccine Immunol. **17:**1734–1738. http://dx.doi.org/10.1128/CVI.00332-10.
7. **Binnicker MJ, Jespersen DJ, Harring JA, Rollins LO, Beito EM.** 2008. Evaluation of a multiplex flow immunoassay for detection of Epstein-Barr virus-specific antibodies. Clin. Vaccine Immunol. **15:**1410–1413. http://dx.doi.org/10.1128/CVI.00082-08.
8. **Gomez E, Jespersen DJ, Harring JA, Binnicker MJ.** 2010. Evaluation of the Bio-Rad BioPlex 2200 syphilis multiplex flow immunoassay for the detection of IgM- and IgG-class antitreponemal antibodies. Clin. Vaccine Immunol. **17:**966–968. http://dx.doi.org/10.1128/CVI.00086-10.
9. **Jani IV, Janossy G, Brown DW, Mandy F.** 2002. Multiplexed immunoassays by flow cytometry for diagnosis and surveillance of infectious diseases in resource-poor settings. Lancet Infect. Dis. **2:**243–250. http://dx.doi.org/10.1016/S1473-3099(02)00242-6.
10. **Kaul R, Chen P, Binder SR.** 2004. Detection of immunoglobulin M antibodies specific for *Toxoplasma gondii* with increased selectivity for recently acquired infections. J. Clin. Microbiol. **42:**5705–5709. http://dx.doi.org/10.1128/JCM.42.12.5705-5709.2004.
11. **Franck J, Garin YJ, Dumon H.** 2008. LDBio-Toxo II immunoglobulin G Western blot confirmatory test for anti-*Toxoplasma* antibody detection. J. Clin. Microbiol. **46:**2334–2338. http://dx.doi.org/10.1128/JCM.00182-08.
12. **Jost C, Touafek F, Fekkar A, Courtin R, Ribeiro M, Mazier D, Paris L.** 2011. Utility of immunoblotting for early diagnosis of toxoplasmosis seroconversion in pregnant women. Clin. Vaccine Immunol. **18:**1908–1912. http://dx.doi.org/10.1128/CVI.05303-11.
13. **Villard O, Breit L, Cimon B, Franck J, Fricker-Hidalgo H, Godineau N, Houze S, Paris L, Pelloux H, Villena I, Candolfi E.** 2013. Comparison of four commercially available avidity tests for *Toxoplasma gondii*-specific IgG antibodies. Clin. Vaccine Immunol. **20:**197–204. http://dx.doi.org/10.1128/CVI.00356-12.
14. **Morgan E, Varro R, Sepulveda H, Ember JA, Apgar J, Wilson J, Lowe L, Chen R, Shivraj L, Agadir A, Campos R, Ernst D, Gaur A.** 2004. Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. Clin. Immunol. **110:**252–266. http://dx.doi.org/10.1016/j.clim.2003.11.017.
15. **Varro R, Chen R, Sepulveda H, Apgar J.** 2007. Bead-based multianalyte flow immunoassays: the cytometric bead array system. Methods Mol. Biol. **378:**125–152. http://dx.doi.org/10.1007/978-1-59745-323-3_9.
16. **Viera AJ, Garrett JM.** 2005. Understanding interobserver agreement: the kappa statistic. Fam. Med. **37:**360–363.
17. **Remington JS, Thulliez P, Montoya JG.** 2004. Recent developments for diagnosis of toxoplasmosis. J. Clin. Microbiol. **42:**941–945. http://dx.doi.org/10.1128/JCM.42.3.941-945.2004.
18. **Montoya JG, Remington JS.** 2008. Management of *Toxoplasma gondii* infection during pregnancy. Clin. Infect. Dis. **47:**554–566. http://dx.doi.org/10.1086/590149.
19. **Pignanelli S.** 2011. Laboratory diagnosis of *Toxoplasma gondii* infection with direct and indirect diagnostic techniques. Indian J. Pathol. Microbiol. **54:**786–789.