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Evaluation of the Liaison Automated Testing System for Diagnosis of Congenital Toxoplasmosis

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Congenital toxoplasmosis is a worldwide health problem, and different screening strategies exist. Testing of toxoplasma-specific antibodies in infants identifies congenital toxoplasmosis during the first year of life. However, experience with commercial available immunoassays is limited. The aim of this study was to evaluate both the performance and analytical characteristics of the Liaison diagnostic system in infants. In a retrospective study, serum *Toxoplasma gondii* antibodies were measured in samples from 333 infants, including 212 noninfected infants and 121 infants with congenital toxoplasmosis. A total of 1,157 umbilical cord blood and peripheral serum samples were analyzed. Liaison toxoplasma-specific IgG and IgM antibodies and the IgG avidity index were compared to the infection status of the infant, determined by the Sabin-Feldman dye test and immunosorbent agglutination assay—IgM. All noninfected infants were seronegative by Liaison IgG within the first year of life. The Liaison system showed a sensitivity of 81.8%, a specificity of 100.0%, a positive predictive value of 100.0%, a negative predictive value of 90.6%, and overall agreement of 84.4% by comparison with the dye test. Overall agreement of both IgM test systems was 96.0%. In this study cohort, avidity did not show a potential diagnostic benefit for the detection of congenital infection. In conclusion, the Liaison system is a valuable tool to monitor the serologic course of infants at risk. A final serologic confirmatory test is recommended to improve the rate of detection of congenital toxoplasmosis at 1 year of life. Protocols of routine follow-up testing in infants and accurate diagnostic tools after acute gestational infections are needed to improve medical care.

Infection with the parasite *Toxoplasma gondii* is a common disease and a major public health problem worldwide, especially in immunocompromised/immunodeficient patients and pregnant women (16). Seroprevalence ranges from less than 20% in northern Europe to more than 60% in southern Europe (38). Primary infection in pregnant women is typically asymptomatic. Therefore, only serologic screening detects acute infection in pregnant women accurately, because unrecognized infection can be transmitted to the unborn (37). In Europe, the overall transmission risk during pregnancy is approximately 29%, and the risk increases to 72% when maternal acute infection occurs at the end of pregnancy (6). Rapid treatment following acute maternal infection efficiently reduces the transmission risk and the clinical burden in the infant (14). Furthermore, the frequency and severity of congenital infection vary depending on virulence of the parasite strain, the mother's immune response, and placental permeability (32).

Congenital infection may cause a broad spectrum of clinical presentation, such as retinochorioiditis, cerebral calcifications, hydrocephalus, mental retardation, and death (20, 30). Infected infants may also present in about 72% of cases without symptoms at birth, and thus their infection is often not recognized at birth, with the risk of severe sequelae in later life, serious neurological sequelae in 8% of cases or ocular disease in 18%, respectively (4, 10, 34, 36).

In Austria, pregnant women are tested for toxoplasma infections by means of a nationwide routine serologic screening program (1). The major goal of this prenatal screening program is to identify pregnant women with acute infection and consequently fetuses at risk of congenital infection. In the case of a proven toxoplasma infection during pregnancy, a comprehensive, standardized, serological, and clinical program of follow-up of the offspring is available. Noninfected infants are monitored until seronegativity (IgG) is determined, and infants with congenital toxoplasmosis are examined annually. In infants with congenital toxoplasmosis, antiparasitic treatment is recommended during the first year of life; therefore, the accurate diagnosis is essential (28).

The Sabin-Feldman dye test (DT), still considered the "gold standard method" for the detection of toxoplasma infections, is expensive and time-consuming (29). Its application is therefore restricted to specialized laboratories as a confirmatory test, and it serves as a standard for validation of new test systems (13, 17, 27). However, DT is scarcely available in most countries, and commercial automated test systems for postnatal routine serologic screening during the first year of life to discriminate congenital and noninfected infants are needed. The Liaison testing system had already been evaluated in pregnant women (23), but no data for the serologic profile in infants are available. The aim of the study was to evaluate the Liaison diagnostic system for toxoplasma-specific IgM and IgG antibodies and IgG avidity (DiaSorin, Saluggia, Italy) for the analysis of umbilical cord or peripheral blood samples of infants with risk of materno-fetal transmission. The results of the Liaison system were compared to those for DT and immunosorbent agglutination assay (ISAGA)-IgM in the Toxoplasmosis Reference Center, Medical University of Vienna, Austria, and

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the serologic courses of 212 untreated noninfected and 121 treated congenitally infected infants, including clinical outcomes, in the first 12 months of life.

MATERIALS AND METHODS

In this study, serum samples from offspring of women with proven seroconversion detected by the routine Austrian toxoplasmosis screening program were included. Infants were serologically and clinical monitored during the first year of life by the Toxoplasmosis Reference Center, Medical University of Vienna, Vienna, Austria. The examination schedule for the infants was at least at birth and at 3-month intervals during the first year of life and annually in case of congenital infection. All infants were followed up for at least 12 months. The mean interval of follow-up of noninfected infants was 13 months, and that of congenital infected infants was 31 months. Infected infants received antibiotic treatment during the first year of life. Infants were classified according to Lebech criteria in group 1, noninfected infants (NI), seronegativity within the first year of life; and group 2, infants with congenital toxoplasmosis (CT), IgG persistence after the first year of life as determined by DT (19).

A total of 1,157 sera (181 umbilical cord blood samples at birth and 976 peripheral blood samples) were analyzed by the Liaison testing system. Group 1 included 613 sera (129 umbilical cord and 484 peripheral blood samples) from 212 infants, and group 2 included 544 sera (52 umbilical cord and 492 peripheral blood samples) from 121 infants.

Routine laboratory analyses were performed, including in-house DT and ISAGA-IgM (bioMérieux, France) within 24 to 48 h after arrival, as previously described (2, 5, 11, 33). The samples were shipped at room temperature. Aliquots of sera were stored at −20°C, and for the evaluation of the Liaison toxoplasma-specific assays, aliquots of sera were thawed and retrospectively analyzed.

The in-house method DT included the measurement of all immunoglobulin subclasses simultaneously (cutoff, 1:4 positive). Interpretation of ISAGA-IgM results was done according to the manufacturer's recommendations (index, <3, negative; ≥3, positive). The study included testing of toxoplasma-specific IgG and IgM antibodies and IgG avidity by the Liaison diagnostic system (DiaSorin, Saluggia, Italy). This immunoassay uses magnetic microparticles coated with inactivated *Toxoplasma gondii* (RH strain) and monoclonal antibodies labeled with an isoluminol derivative. The test principle of performing the analysis of Liaison toxoplasma-specific IgG and IgM antibodies and IgG avidity was described elsewhere (23).

Liaison cutoff values. For IgG, the measurement range is 0 to 400 IU/ml. The cutoff value for positive results is above 8.8 IU/ml, borderline results range from 7.2 to 8.8 IU/ml, and negative results are defined as values below 7.2 IU/ml. For IgM, the measurement range is between 0 and 160 arbitrary units (AU)/ml. Positive results are defined as those above 8 AU/ml, borderline results are from 6 to 8 AU/ml, and negative results are below 6 AU/ml. The IgG avidity index ranges from 0.01 to 0.95. Avidity is classified as low (avidity index < 0.3), moderate (avidity index 0.3 to 0.4), or high (avidity index > 0.4) avidity. All cutoff values were used according to the manufacturer's recommendation.

Statistics. Calculations were performed by using the software programs Excel 2007 (Microsoft, Redmond, WA), SPSS 16.0 (SPSS Science, Chicago, IL), and MedCalc 9.0 (MedCalc Software, Belgium) for the receiver operating characteristics (ROC) analysis. 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 infection status of the child, respectively.

RESULTS

Performance of the Liaison IgG assay. A total of 1,157 samples were analyzed for toxoplasma-specific IgG levels. The overall agreement of DT versus both Liaison testing systems was 84.4%, with an *r* value of 0.580 and a kappa of 0.504.

In group 1, a total of 613 specimens from 212 noninfected infants were analyzed. Sera from 83 infants were excluded due to long test intervals ($n = 77$) or equivocal test results ($n = 6$). Finally, 476 specimens (umbilical cord blood samples, $n = 80$; peripheral blood sera, $n = 396$) from 129 infants with complete serologic course were analyzed. Liaison IgG levels were reported as negative for 12/129 (9.3%) infants within 3 months, 52/129 (40.3%) within 6 months, 35/129 (27.1%) within 9 months, and the remaining 30/129 (23.3%) within the first 12 months of life. In conclusion, all Liaison IgG levels were reported as negative in noninfected infants within 1 year.

Group 2 comprised 544 sera from 121 infants with congenital toxoplasmosis. In this group, a total of 20/544 (3.7%) sera were excluded (long test intervals or only a single specimen). From the remaining 106 infected infants, 494/524 (94.3%) specimens were positive, and 30/524 (5.7%) sera tested negative for 22 infected infants by the Liaison IgG test system. A detailed overview is given in Table 1, including Liaison IgG and IgM in comparison to DT and ISAGA-IgM values.

ROC for Liaison IgG cutoff determination. We analyzed Liaison IgG levels with the cutoff level according to the manufacturer's recommendation (IgG positive, >8.8 IU/ml) compared to the infection status of the child (noninfected versus congenitally infected). In addition, ROC were calculated to improve sensitivity and specificity for different cutoff values according to the time point of sampling (at 6, 9, and 12 months of life). Using a cutoff value of >8.8 IU/ml, the highest sensitivity (96.4%; 95% confidence interval [CI], 89.9 to 99.2) but lowest specificity (37.8%; 95% CI, 30.5 to 45.5) were observed at 6 months of life (Table 2). In comparison, a sensitivity of 75.0% (95% CI, 63.0 to 84.7) and specificity of 96.7% (95% CI, 90.7 to 99.3) were calculated for 12 months of life. Decreasing the cutoff value from >8.8 to around 5 IU/ml did not result in a substantial benefit in increasing either the sensitivity or the specificity of the Liaison IgG assay. In conclusion, a modification of the recommended cutoff in pediatric specimen did not improve the performance of the system to discriminate noninfected from infected infants in this study.

Performance of the Liaison IgM assay. All samples were tested using the Liaison toxoplasma-specific IgM assay, and results were compared to those for ISAGA-IgM (cutoff for infants ≥ 3). Table 3 shows the comparison of results for 1,157 samples (umbilical cord blood, $n = 181$; peripheral blood sera, $n = 976$) measured with both test systems. The results were displayed according to the infection status of the infant (Table 3). In umbilical cord blood, nine false-positive IgM test results were reported for noninfected infants. The overall agreement of both test systems was 96.0% ($r = 0.593$ and kappa = 0.582).

Performance of Liaison IgG avidity index. The analysis of the Liaison IgG avidity index was performed for 518 samples (NI, $n = 285$; CT, $n = 233$) and compared to the time point of sampling at 0 to 3, >3 to 6, >6 to 9, and >9 to 12 months of life. We observed a statistically significant difference at the latter time point of sampling ($P = 0.003$), and three of nine samples had a high avidity index in the noninfected group 1, in contrast to 11of 50 that had a low index in group 2.

DISCUSSION

The accurate diagnosis of congenital toxoplasmosis, in order to avoid serious sequelae, is still challenging, and protocols for serological follow-up are mandatory. Reasons are, e.g., that the production of antibodies against *Toxoplasma gondii* in the fetus

TABLE 1 Detailed overview of 22 infants with congenital toxoplasmosis and negative Liaison IgG result

Infant ID[a]	Age (mo) at negative Liaison IgG result(s)	Assay result(s)				Comment on Liaison results
		Liaison IgG (IU/ml)	Liaison IgM (AU/ml)	DT	ISAGA-IgM (index)	
1	6	4.5	<3.0	1,024	0	IgG positive at 1, 4, 5 mo
2	12	3.8	<3.0	4	0	IgG positive at 6 and 57 mo
3	13	<3.0	<3.0	4	0	IgG positive at 3, 6, 19 mo
4	11, 13	3.0, 2.0	<3.0	4	0	IgG positive at 1, 4 mo and 29, 56, 66, 92, 102, 117, 131 mo
5	12	6.8	<3.0	64	2	IgG positive at 8 mo and 25, 48 mo
6	175	6.1	<3.0	64	0	IgG positive at 1, 5, 11 mo
7	9	<3.0	<3.0	4	0	IgG positive at 20, 37 mo
8	9, 12, 13	7.1, 2.0, <3.0	6.1, <3.0, <3.0	16, 4, 4	0	IgG positive at 0, 3, and 37 mo
9	15	5.2	<3.0	64	0	IgG positive at 1, 3, 4, 7, 9 and 26 mo; equivocal (IgG, 8.5) at 12 mo
10	9	6.0	<3.0	64	0	IgG positive at 49, 60, 73 mo
11	12	<3.0	<3.0	4	0	IgG positive at 0 and 33 mo
12	11	2.0	3.0	4	0	IgG positive at 5 and 15 (also IgM positive), 28, 43 mo
13	14	3.5	3.8	64	1	IgG positive at 19, 33, 66, 80 mo
14	11	4.0	<3.0	16	0	IgG positive at 0, 3 mo
15	7, 10, 14	<3.0, <3.0, 3.2	<3.0, <3.0, 5.4	16, 16, 64	0	IgG positive at 0, 4 mo and 27, 37, 57 mo
16	9	6.4	<3.0	256	0	IgG positive at 5, 6 and 13 (also IgM positive), 15 mo
17	10	5.6	<3.0	16	0	IgG positive at 3, 6 and 15, 24 mo
18	7	2.0	<3.0	16	0	IgG positive at 3 and 34, 62, 77, 96 mo
19	12	2.0	<3.0	4	0	IgG positive at 0, 3, 7 and 16 (also IgM positive) mo
20	41, 55, 65	3.4, <3.0, <3.0	<3.0	64, 16, 16	0	IgG positive at 3, 6, 9, 14, 28 and 102, 128 mo
21	30	<3.0	<3.0	16	0	IgG positive at 8 mo; equivocal (IgG, 8.1) at 15 mo
22	8, 12	5.9, <3.0	<3.0	256, 16	0	IgG positive at 3, 6 mo

[a] Identification no.

or newborn is often inhibited and/or masked by maternal antibodies (31). Early diagnosis is important because the initiation of antibiotic treatment in infected infants is mandatory soon after birth (4, 8). Different approaches exist, such as PCR diagnostics, genotyping, comparative IgG profiles between mother and child, serological typing, and Western blot analyses (9, 12, 15, 18, 22). These methods are supporting, but the first-line screening method used by routine clinical laboratories to confirm or exclude congenital infection is still serology. Offspring of mothers with acute gestational toxoplasma infection have to be included in a follow-up program. Umbilical cord blood analysis at birth and subsequent peripheral blood testing every 3 months during the first year of life are recommended. At least at the end of the first year, the diagnosis has to be determined. Little is known about serologic profiles of infants obtained by different testing systems (7, 21, 24, 25).

TABLE 2 Sensitivity and specificity for different Liaison IgG cutoff values for differentiation in noninfected and congenitally infected children at 6, 9, and 12 months of life

Time point (mo of life) for analysis of peripheral blood samples	Cutoff value used (IU/ml)	Sensitivity (%)	95% CI	Specificity (%)	95% CI
6[a]	>4.5	97.6	91.6–99.6	23.3	17.2–30.3
	>8.8[d]	96.4	89.9–99.2	37.8	30.5–45.5
9[d]	>5.6	94.6	84.9–98.8	78.4	68.8–86.1
	>8.8[d]	87.3	75.5–94.7	87.6	79.4–93.4
12[c]	>5.0	79.4	67.9–88.3	96.7	90.7–99.3
	>8.8[d]	75.0	63.0–84.7	96.7	90.7–99.3

[a] NI group, $n = 172$; CT group, $n = 84$.
[b] NI group, $n = 97$; CT group, $n = 55$.
[c] NI group, $n = 91$; CT group, $n = 68$.
[d] According to the manufacturer's recommendation.

TABLE 3 Comparison of Liaison-IgM and ISAGA-IgM results according to infection status of infants

Infection status	No. of samples with result[a]					
	IgM negative		IgM borderline		IgM positive	
	ISAGA	Liaison	ISAGA	Liaison	ISAGA	Liaison
No infection	599 (CB, 119)	603 (CB, 120)		4 (CB, 3)	14 (CB, 10)	6 (CB, 6)
Congenital infection with *Toxoplasma gondii*	484 (CB, 32)	488 (CB, 36)		16 (CB, 1)	60 (CB, 20)	40 (CB, 15)
Total	1,083 (CB, 151)	1,091 (CB, 156)		20 (CB, 4)	74 (CB, 30)	46 (CB, 21)

[a] CB, cord blood samples; ISAGA, immunosorbent agglutination assay—IgM.

The aim of the study was to compare the Liaison toxoplasma-specific assays with the DT and ISAGA-IgM. The objective was to evaluate the test performance and characteristics. Hereby, we investigated for the first time the Liaison diagnostic system to monitor the course of toxoplasma-specific immunoglobulins in infants.

As reported recently, isolated immunoglobulin levels in immunoassays should not be compared solely to results of DT because this method detects all classes of immunoglobulins simultaneously, and due to this fact, DT is more sensitive (23, 26). However, we observed a good overall agreement for both the Liaison IgG (84.4%) and IgM (96.0%) assays. Even though all noninfected infants were reported Liaison IgG negative within 1 year of life, our study reported seronegative peripheral blood samples by the Liaison system for the congenitally infected group. Consequently, we recommend a final serologic confirmatory analysis in a reference laboratory in terms of seronegative results to exclude congenital infection after the first year of life. This is a common issue of immunoassays compared to DT in terms of immunoglobulin levels produced in concentrations below the sensitivity thresholds of the methods available. Our study revealed 22 congenitally infected infants with IgG seronegativity in the Liaison (Table 1) and seropositivity in the Sabin-Feldman dye test. Furthermore, in subsequent samples the infants were seropositive, but this was not a serological rebound but rather a lack of sensitivity (3, 35). In particular, a diminished sensitivity of the Liaison IgG was observed between 6 and 12 months of life due to the decrease of maternally diaplacental antibodies against *Toxoplasma gondii*. We attempt to optimize IgG cutoff values by ROC curve analyses, but this did not result in any improvement. Consequently, changing the manufacturer's cutoff value for infants suspected of having congenital infection cannot be recommended.

The sensitivity of using IgM for neonatal screening was reported to be about 50%, and thus it cannot be used as a single parameter in newborns depending on the testing system and the gestational age of maternal seroconversion (11). However, our study revealed Liaison-IgM-positive umbilical cord blood samples for noninfected infants. We hypothesize that these false-positive results were due to contamination during blood sampling. Hence, any reported positive IgM test for umbilical cord blood should be subsequently verified using peripheral blood. Nonetheless, increased IgM levels detected in peripheral blood during the first year of life strongly indicate congenital infection, and therefore, IgM analysis in infants of risk is recommended routinely during the first year of life.

In adults, avidity enables the determination of an estimated time point of infection. A low index was found for many patients even more than 4 months after infection (23). In contrast, we observed a higher avidity index for the infected infants (group 2) between 9 and 12 months, but these findings could not be used for early diagnosis in this study cohort.

In conclusion, commercially available immunoassays have been widely used for adults, but experience in determination of the serological course of children at risk is still limited. We present the first evaluation of the Liaison diagnostic system to monitor the serologic course of infection in infants from mothers with acute gestational toxoplasma infection. Both IgG and IgM levels should be monitored at 3-month intervals for at least 1 year of life or until seronegativity. During the first year of life, all noninfected infants showed a decrease in the IgG concentration. However, we observed a lack of sensitivity for seropositivity of congenitally infected children. Finally, we recommend a serologic confirmatory testing in a reference laboratory in terms of seronegative results within 1 year of life. This test system provides a method for serologic follow-up programs for offspring to improve medical care.

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REFERENCES

1. **Aspock H, et al.** 1994. Toxoplasmosis. Recommendations for treatment of primary toxoplasma infection in pregnancy and congenital toxoplasmosis. Gynakol. Geburtshilfliche Rundsch. **34**:50–51. (In German.)
2. **Bessieres MH, et al.** 2009. Diagnosis of congenital toxoplasmosis: prenatal and neonatal evaluation of methods used in Toulouse University Hospital and incidence of congenital toxoplasmosis. Mem. Inst. Oswaldo Cruz **104**:389–392.
3. **Ciardelli L, et al.** 2008. Early and accurate diagnosis of congenital toxoplasmosis. Pediatr. Infect. Dis. J. **27**:125–129.
4. **Cortina-Borja M, et al.** 2010. Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. PLoS Med. **7**:e1000351. doi:10.1371/journal.pmed.1000351.
5. **Desmonts G, Naot Y, Remington JS.** 1981. Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired Toxoplasma infections. J. Clin. Microbiol. **14**:486–491.
6. **Dunn D, et al.** 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet **353**:1829–1833.
7. **Foudrinier F, et al.** 2003. Clinical value of specific immunoglobulin E detection by enzyme-linked immunosorbent assay in cases of acquired and congenital toxoplasmosis. J. Clin. Microbiol. **41**:1681–1686.
8. **Foulon W, et al.** 1999. Treatment of toxoplasmosis during pregnancy: a multicenter study of impact on fetal transmission and children's sequelae at age 1 year. Am. J. Obstet. Gynecol. **180**:410–415.
9. **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.

10. **Freeman K, et al.** 2008. Predictors of retinochoroiditis in children with congenital toxoplasmosis: European, prospective cohort study. Pediatrics **121**:e1215–22. doi:10.1542/peds.2007-2169.
11. **Gilbert RE, et al.** 2007. Screening for congenital toxoplasmosis: accuracy of immunoglobulin M and immunoglobulin A tests after birth. J. Med. Screen. **14**:8–13.
12. **Gross U, et al.** 2000. Comparative immunoglobulin G antibody profiles between mother and child (CGMC test) for early diagnosis of congenital toxoplasmosis. J. Clin. Microbiol. **38**:3619–3622.
13. **Hayde M, Salzer HR, Gittler G, Aspock H, Pollak A.** 1995. Microparticle enzyme immunoassay (MEIA) for toxoplasma specific immunoglobulin G in comparison to the Sabin-Feldman dye test. A pilot study. Wien. Klin. Wochenschr. **107**:133–136.
14. **Hotop A, Hlobil H, Gross U.** 2012. Efficacy of rapid treatment initiation following primary Toxoplasma gondii infection during pregnancy. Clin. Infect. Dis. **54**:1545–1552.
15. **Jalal S, Nord CE, Lappalainen M, Evengard B.** 2004. Rapid and sensitive diagnosis of Toxoplasma gondii infections by PCR. Clin. Microbiol. Infect. **10**:937–939.
16. **Joynson DH, Wreghitt TJ.** 2001. Toxoplasmosis: a comprehensive clinical guide. Cambridge University Press, Cambridge, England.
17. **Kasper DC, et al.** 2009. Evaluation of the Vitros ECiQ immunodiagnostic system for detection of anti-Toxoplasma immunoglobulin G and immunoglobulin M antibodies for confirmatory testing for acute Toxoplasma gondii infection in pregnant women. J. Clin. Microbiol. **47**:164–167.
18. **Kasper DC, et al.** 2009. Quantitative real-time polymerase chain reaction for the accurate detection of Toxoplasma gondii in amniotic fluid. Diagn. Microbiol. Infect. Dis. **63**:10–15.
19. **Lebech M, et al.** 1996. Classification system and case definitions of Toxoplasma gondii infection in immunocompetent pregnant women and their congenitally infected offspring. European Research Network on Congenital Toxoplasmosis. Eur. J. Clin. Microbiol. Infect. Dis. **15**:799–805.
20. **Montoya JG, Liesenfeld O.** 2004. Toxoplasmosis. Lancet **363**:1965–1976.
21. **Naessens A, et al.** 1999. Diagnosis of congenital toxoplasmosis in the neonatal period: A multicenter evaluation. J. Pediatr. **135**:714–719.
22. **Nowakowska D, et al.** 2006. Genotyping of Toxoplasma gondii by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis. J. Clin. Microbiol. **44**:1382–1389.
23. **Petersen E, et al.** 2005. European multicenter study of the LIAISON automated diagnostic system for determination of Toxoplasma gondii-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. J. Clin. Microbiol. **43**:1570–1574.
24. **Pinon JM, et al.** 1996. Early neonatal diagnosis of congenital toxoplasmosis: value of comparative enzyme-linked immunofiltration assay immunological profiles and anti-Toxoplasma gondii immunoglobulin M (IgM) or IgA immunocapture and implications for postnatal therapeutic strategies. J. Clin. Microbiol. **34**:579–583.
25. **Pinon JM, et al.** 2001. Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. J. Clin. Microbiol. **39**:2267–2271.
26. **Press C, Montoya JG, Remington JS.** 2005. Use of a single serum sample for diagnosis of acute toxoplasmosis in pregnant women and other adults. J. Clin. Microbiol. **43**:3481–3483.
27. **Prusa AR, et al.** 2010. Evaluation of the Roche Elecsys Toxo IgG and IgM electrochemiluminescence immunoassay for the detection of gestational Toxoplasma infection. Diagn. Microbiol. Infect. Dis. **68**:352–357.
28. **Prusa AR, Hayde M, Gratzl R.** 2000. Connatale Toxoplasmose und connatale Toxoplasma-Infektion. Pädiatr. Pädol. **6**:39–42.
29. **Reiter-Owona I, et al.** 1999. The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. Bull. World Health Organ. **77**:929–935.
30. **Remington JS, McLeod R, Thulliez P, Desmonts G.** 2000. Toxoplasmosis, p 205–346. *In* Remington JS, Klein JO (ed), Infectious diseases of the fetus and newborn infant, 5th ed. W. B. Saunders Company, Philadelphia, PA.
31. **Robert-Gangneux F, et al.** 1999. Value of prenatal diagnosis and early postnatal diagnosis of congenital toxoplasmosis: retrospective study of 110 cases. J. Clin. Microbiol. **37**:2893–2898.
32. **Robert-Gangneux F, et al.** 2011. The placenta: a main role in congenital toxoplasmosis? Trends Parasitol. **27**:530–536.
33. **Sabin AB, Feldman HA.** 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science **108**:660–663.
34. **Soares JA, Carvalho SF, Caldeira AP.** 2012. Profile of pregnant women and children treated at a reference center for congenital toxoplasmosis in the northern state of Minas Gerais, Brazil. Rev. Soc. Bras Med. Trop. **45**:55–59.
35. **Wallon M, Cozon G, Ecochard R, Lewin P, Peyron F.** 2001. Serological rebound in congenital toxoplasmosis: long-term follow-up of 133 children. Eur. J. Pediatr. **160**:534–540.
36. **Wilson CB, Remington JS, Stagno S, Reynolds DW.** 1980. Development of adverse sequelae in children born with subclinical congenital Toxoplasma infection. Pediatrics **66**:767–774.
37. **Wong SY, Remington JS.** 1994. Toxoplasmosis in pregnancy. Clin. Infect. Dis. **18**:853–861; quiz, 862.
38. **Zuber P, Jacquier P.** 1995. Epidemiology of toxoplasmosis: worldwide status. Schweiz. Med. Wochenschr. Suppl. **65**:19S–22S. (In French.)