JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2005, p. 1570–1574 Vol. 43, No. 4
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.4.1570–1574.2005

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

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Received 5 June 2004/Returned for modification 16 August 2004/Accepted 24 November 2004

The LIAISON system is a fully automated system based on chemiluminescence and antigen bound to magnetic microparticles. The system allows fast and precise measurement of *Toxoplasma*-specific immunoglobulin G (IgG) and IgM antibody levels and measurement of the IgG avidity index even at low levels of *Toxoplasma*-specific IgG antibodies in a single step without manual interference. Seven European centers participated in a multicenter evaluation of the LIAISON system. The sensitivity and specificity of the LIAISON system compared to the Sabin-Feldman dye test were 99.3 and 96.8%, respectively. In a comparison of the LIAISON *Toxoplasma*-specific IgM assay with an immunosorbent agglutination assay, the LIAISON assay had a sensitivity of 96.7% and a specificity of 95.4%. The LIAISON IgG assay showed agreements of 91, 100, and 100% with the AXSYM IgG (Abbott), VIDAS IgG (bioMérieux), and Platelia IgG (Bio-Rad) assays, respectively. The LIAISON IgM assay showed agreements of 95% with the AXSYM IgM and Platelia IgM assays, 96% with the ISAGA IgM assay (bioMérieux), and 97% with the VIDAS IgM assay. The coefficient of correlation between the LIAISON system and the VIDAS *Toxoplasma*-specific IgG avidity index was 0.81. By use of the *Toxoplasma*-specific IgG avidity index assay with specific IgM-positive samples, the diagnosis of infection with *Toxoplasma gondii* in early pregnancy has been improved significantly. The LIAISON avidity assay is a valuable assay for the exclusion of recently acquired infection with *T. gondii* (less than 4 months) in pregnant women, and it decreases significantly the necessity for follow-up testing.

Primary maternal infection with *Toxoplasma gondii* carries the risk of transmitting the infection to the fetus, resulting in congenital infection. Congenital infection of the fetus in women infected just before conception is extremely rare, and even during the first few weeks of pregnancy, the maternal-fetal transmission rate is low (6). It is therefore essential to estimate the time of infection as precisely as possible to properly manage the risk to the fetus of a maternal infection. Low levels of *Toxoplasma*-specific immunoglobulin M (IgM) antibodies may be found for up to several years, and the mere demonstration of low levels of *Toxoplasma*-specific IgM antibodies is therefore not a sign by itself of acute infection with *T. gondii* (16, 17). The measurement of the avidity of IgG antibodies for *T. gondii* infections was first demonstrated in 1989 (8, 9, 15) and since then has been further developed (4, 18).

A study of the diagnostic value of various tests for acute infections with *T. gondii*, including *Toxoplasma*-specific IgG, IgM, and IgA antibodies and the IgG avidity index, showed that the combination of a sensitive test for *Toxoplasma*-specific IgM antibodies and a *Toxoplasma*-specific IgG avidity assay had the highest predictive value with regard to the time of infection (23).

Since previous studies showed that some individuals have low-avidity IgG antibodies many months after infection, we also tested the hypothesis of whether treatment influences the maturation of IgG antibodies.

The LIAISON diagnostic system (DiaSorin, Saluggia, Italy) is the first fully automated immunoassay based on chemiluminescence and antigen bound to magnetic microparticles. It is the first assay to allow measurement of the *Toxoplasma*-specific IgG avidity index at low levels of specific IgG antibodies.

The aim of this study was to validate the LIAISON system in European reference laboratories with the specific aims of validating the automated measurement of the *Toxoplasma*-specific IgG avidity index and to study whether treatment influences the maturation of the IgG response.

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METHODS AND MATERIALS

Samples and patients. Each center contributed samples according to the categories of patients available; in total, 1,488 samples were included. Each center had a LIAISON system available in the participating laboratories for the

study. Centers involved in prenatal screening contributed samples from women showing seroconversion. All samples were randomized. The test populations were defined by how the centers performed screening, as follows: prenatal screening every month (Paris and Pavia), screening every 3 months during pregnancy (Brussels and Seville), neonatal screening (Copenhagen), and no screening (Berlin and Swansea). In centers with no prenatal screening, populations were defined based on their profiles in various serological tests.

(i) Seville, Spain. A total of 157 samples were tested with the LIAISON system for *Toxoplasma*-specific IgG and IgM antibodies and the IgG avidity index. These included 41 samples from patients with acute infections and a known time of seroconversion within the preceding 4 months, 78 samples from patients in the convalescence phase (infections acquired between 4 and 12 months before), 25 samples from patients in the latent phase (positive for *Toxoplasma*-specific IgG for at least 12 months), and 13 samples from *Toxoplasma*-seronegative patients.

(ii) Berlin, Germany. The samples tested included 20 samples from patients with recent infections (high Sabin-Feldman dye test result, positive for IgM, and low avidity index), 13 samples from patients who were positive for IgG and IgM and had a high avidity index, 14 samples from patients who were positive for IgG and negative for IgM, and 92 samples from IgG- and IgM-negative patients. The samples were tested with the AXSYM system (Abbott, Chicago, Ill.) (5), the VIDAS system (bioMérieux, Marcy l'Etoile, France) (19), and the Sabin-Feldman dye test.

(iii) Brussels, Belgium. The samples tested included 67 samples from women with acute infections and a known time of seroconversion, 38 samples from patients with previous infections and no IgM, 43 samples from patients who had a low level of IgG and who were positive for *Toxoplasma*-specific IgM antibodies, and 33 samples from seronegative patients. Routine testing was performed by use of assays from Bio-Rad (Marnes-la-Coquette, France) (12).

(iv) Copenhagen, Denmark. A total of 291 consecutive samples submitted from hospitals and general practitioners were tested with the VIDAS and LIAISON systems for *Toxoplasma*-specific IgG and IgM antibodies and the IgG avidity index.

(v) Paris, France. A total of 435 samples were used in the study. These included 67 samples obtained from patients with acute infections within 2 to 6 months after infection onset, 62 serial samples from 17 patients who had seroconverted, 160 samples from patients with past infections (80 of which had persistent IgM antibodies and 80 of which were negative for IgM), 120 samples from seronegative patients, and 26 samples from 9 patients with previous infections and showing a serological reactivation. Routine testing was performed with the VIDAS enzyme-linked immunosorbent assay, the VIDAS IgG avidity test, the ISAGA IgM assay (bioMérieux), the Sabin-Feldman dye test, and the differential agglutination assay (4, 7).

(vi) Pavia, Italy. A total of 55 consecutive samples from 17 patients with acute infections, 32 samples from patients with recent infections, 62 samples from patients with past infections, and 94 samples from seronegative patients were included. Twenty samples from individuals who were not pregnant and who were not treated were also included. Routine testing was performed with the VIDAS IgG assay and IgG avidity assay, the ISAGA IgM assay, and the direct agglutination assay (bioMérieux).

(vii) Swansea, United Kingdom. A total of 181 samples were tested with in-house assays and the LIAISON system for *Toxoplasma*-specific IgG and IgM antibodies and the IgG avidity index. These included 18 samples from patients with acute infections, 90 samples from patients with recent infections (65 of which were tested for the IgG avidity index), 40 samples from patients with past infections, and 33 samples from seronegative patients. Twelve samples from individuals who were not pregnant and who were not treated were also included. The laboratory used in-house enzyme-linked immunosorbent assays for IgG, IgM, and the IgG avidity index.

LIAISON system. The LIAISON immunoassay analyzer is a fully automated random-access analyzer. Its test principle is based on magnetic microparticle technology, chemiluminescence with flash light kinetics, and an isoluminol derivative as a label. The analyzer is a benchtop system with a loading capacity of 144 samples and is designed to handle primary tubes with bar code identification. Continuous reloading is possible, as the samples are placed in 12-tube racks. Samples and reagents are transferred via pipetting needles. The sample volumes used in the assays tested in this study varied between 20 and 30 μl. Reagents required for 100 determinations (LIAISON *Toxoplasma*-specific IgG and LIAISON *Toxoplasma*-specific IgM assays) or 25 determinations (LIAISON *Toxoplasma*-specific IgG avidity assay) are assembled in one integrated reagent cartridge (Reagent Integral) identified by bar-coded labeling, providing information such as parameter information, lot number with expiration date, and recalibration data. The liquid levels of samples, reagents, starter reagents. and system fluid are checked via sensors. The reagents are stored in a cooled, temperature-controlled area and can be continuously reloaded. Up to 15 different kits can be loaded simultaneously. All reagents (magnetic particles, luminescence-labeled tracer, two calibrators, diluent, and assay buffer) are provided ready to use. The walk-away time is at least 6 h. The average throughput is 100 to 140 tests per hour.

The instrument offers variable assay- or patient-specific dilution protocols. The user interface is Windows-based software with both touch screen and keyboard operations. The chemiluminescence signal is generated by the addition of two trigger solutions, and light is quantified as relative light units. Data reduction is based on a master curve with a two-point recalibration method. Calibration allows recalculation of the working standard curve from the stored master standard curve and is performed every 4 weeks with two calibrators in triplicate. A maximum of four calibrations can be performed for every reagent integral. Parameter-specific LIAISON control sera obtained from human serum pools are available from the manufacturer.

***Toxoplasma*-specific IgG antibodies.** The LIAISON *Toxoplasma*-specific IgG assay is completed in 35 min. *Toxoplasma* antigen from a whole-cell lysate is used to coat the solid phase (paramagnetic particles). During the first incubation step, serum or plasma is added to the magnetic particles, incubated for 10 min, and washed three times. A monoclonal IgG antibody to human IgG coupled to an isoluminol derivative is added and incubated with the solid phase for a further 10 min. After washing is done, the chemiluminescence signal is generated. The assay is calibrated against the World Health Organization Third International *Toxoplasma* reference standard, and the measurement range is 0 to 500 IU/ml. Positive results are defined as values above 8 IU/ml, borderline results range from 6 to 8 IU/ml, and negative results are defined as values below 6 IU/ml.

***Toxoplasma*-specific IgM antibodies.** The LIAISON *Toxoplasma*-specific IgM assay is completed in 35 min. A monoclonal IgG antibody to human IgM is used to coat the paramagnetic particles. During the first incubation step, serum or plasma is added to the magnetic particles, incubated for 10 min, and washed. A soluble *Toxoplasma* antigen obtained from a whole-cell lysate is added with a monoclonal IgG antibody to *T. gondii* surface antigen 1 labeled with an isoluminol derivative and incubated with the solid phase for a further 10 min. After washing is done, the chemiluminescence signal is generated. The measurement range is between 0 and 160 arbitrary units (AU)/ml. Positive results are defined as values above 8 AU/ml, borderline results range from 6 to 8 AU/ml, and negative results are defined as values below 6 AU/ml.

***Toxoplasma*-specific IgG avidity index.** The LIAISON *Toxoplasma*-specific IgG avidity assay is completed in 40 min. The avidity index is measured by including a wash step in the IgG test described above; in this step, low-avidity IgG antibodies are eluted from the *Toxoplasma* antigen with 6 M urea buffer. The measurement range is an IgG avidity index of 0.010 and 0.950, calculated as the ratio of the units in the sample treated with 6 M urea buffer to the units in the nontreated sample. The avidity index allows specimen classification as low (avidity index, <0.2), moderate (avidity index, 0.020 to 0.25), or high (avidity index, >0.25) avidity. A high avidity index excludes infections within the previous 4 months.

Statistical analysis. Means were compared with a two-tailed *t* test assuming a normal distribution and independent variance.

RESULTS

The LIAISON system was easy to use, and the integration of the IgG avidity index into the procedure was convenient and time-saving. The LIAISON system is the first system which allows measurement of the IgG avidity index for samples with low levels of IgG antibodies. Not all centers compared the LIAISON system to other systems, and one center compared it to in-house assays.

The Sabin-Feldman dye test measures all immunoglobulin subclasses, and the results of this test therefore cannot be compared to the results of isolated IgG or IgM assays but only to combined IgG and IgM results.

Table 1 shows a comparison of the results of the Sabin-Feldman dye test and the combined IgG and IgM results obtained with the LIAISON system. Assuming that the dye test is the reference standard, the LIAISON system showed a sensitivity of 99.3%, a specificity of 96.8%, a positive predictive value of 98.9%, and a negative predictive value of 98.1% (Ta-

TABLE 1. Comparison of Sabin-Feldman dye test and LIAISON *Toxoplasma*-specific IgG- and IgM assays with 613 samples

Sabin-Feldman dye test result	No. of samples with the following LIAISON IgG and/or IgM assay result:			
	Positive	Equivocal	Negative	Total
Positive	446	3	3	452
Negative	5	3	153	161
Total	451	6	156	613

ble 1). The agreement between the LIAISON system combined IgM and IgG results and the Sabin-Feldman dye test results was 99%.

Table 2 shows a comparison of the LIAISON IgM assay and the ISAGA IgM, VIDAS IgM, AXSYM IgM, and Platelia IgM assays. The agreements were 96% with the ISAGA IgM, 95% with the AXSYM IgM and Platelia IgM, and 97% with the VIDAS IgM assays.

Table 3 shows a comparison of the LIAISON IgG assay and the VIDAS IgG, AXSYM IgG, and Platelia IgG assays. The agreements were 91% with the AXSYM IgG and 100% with both the Platelia IgG and the VIDAS IgG assays.

The LIAISON *Toxoplasma*-specific IgG avidity index was measured for 103 samples from three centers where the date of seroconversion was available. Within the first 4 months, only 2 of 77 samples from pregnant women had an IgG avidity index above 0.25 (Fig. 1). A number of samples continued to show a low IgG avidity index more than 4 months after infection.

The LIAISON *Toxoplasma*-specific IgG avidity index was compared with the results of the VIDAS system, and the correlation is shown in Fig. 2; the correlation coefficient was 0.81.

A total of 32 samples were obtained from individuals who were diagnosed with acute *T. gondii* infection but were not pregnant and therefore were not treated (data not included in Fig. 1). The mean IgG avidity index in treated pregnant women infected within the preceding 16 weeks was 0.092 (standard deviation, 0.047), and that for nontreated nonpregnant individuals was 0.149 (standard deviation, 0.077) (P value determined by a two-tailed t test, 0.0086). There was no significant difference between the two groups at 16 weeks after infection (P value determined by a two-tailed t test, 0.90), but the sample size may have been too small to identify a difference.

TABLE 2. Comparison of LIAISON IgM and other assays for *T. gondii*-specific IgM antibodies

Assay	Cutoff	No. of samples with the following LIAISON IgM assay result:			
		Positive	Equivocal	Negative	Total
ISAGA IgM	9–12	265	6	9	280
	6–8	8	0	0	8
	<6	12	2	249	263
	Total	285	8	258	551
AXSYM IgM	Positive	10	0	2	12
	Equivocal	0	0	0	0
	Negative	3	0	92	95
	Total	13	0	94	107
VIDAS IgM	Positive	39	4	4	47
	Equivocal	0	0	0	0
	Negative	5	3	253	261
	Total	44	7	257	308
Platelia IgM	Positive	61	8	3	72
	Equivocal	0	0	0	0
	Negative	3	1	68	72
	Total	64	9	71	144

TABLE 3. Comparison of LIAISON IgG and other assays for *T. gondii*-specific IgG antibodies

Assay	Cutoff	No. of samples with the following LIAISON IgG assay result:			
		Positive	Equivocal	Negative	Total
AXSYM IgG	Positive	18	1	10	29
	Equivocal	0	0	0	0
	Negative	0	0	89	89
	Total	18	1	99	118
VIDAS IgG	Positive	184	2	1	187
	Equivocal	1	1	0	2
	Negative	0	1	305	306
	Total	185	4	306	495
Platelia IgG	Positive	142	0	0	142
	Equivocal	0	0	0	0
	Negative	0	0	38	38
	Total	142	0	38	180

DISCUSSION

Analysis of *Toxoplasma*-specific IgM and IgG antibodies with the LIAISON IgG and IgM combination showed a good correlation with the dye test. The dye test measures total specific immunoglobulins, including IgG, IgM, and IgA, and the good correlation between the dye test and the LIAISON IgG and IgM combination indicates that the LIAISON *Toxoplasma*-specific IgM assay is able to detect low levels of IgM seen at the beginning of infection.

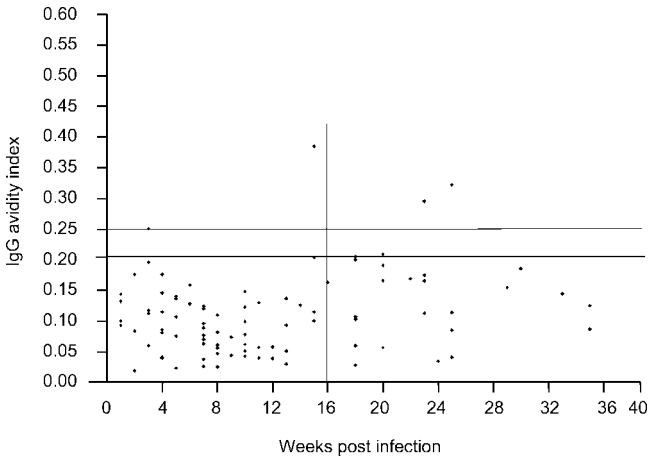

FIG. 1. Development of the LIAISON IgG avidity index in 103 pregnant and treated women monitored after infection (seroconversion) (Brussels, Paris, and Pavia). The cutoff levels are indicated for the IgG avidity index and for the time after infection (see Materials and Methods).

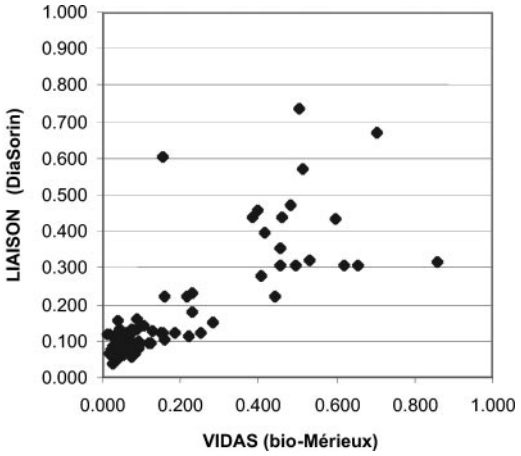

FIG. 2. Correlation ($r = 0.81$) between the *T. gondii*-specific IgG avidity indexes measured by the LIAISON and VIDAS systems.

A comparison with the ISAGA IgM assay showed that nine samples were negative in the LIAISON IgM assay but positive in the ISAGA IgM assay, confirming that the ISAGA IgM assay is more sensitive than the LIAISON IgM assay. It is well known that low levels of *Toxoplasma*-specific IgM antibodies are a common problem in the diagnosis of acute toxoplasmosis (23).

The Sabin-Feldman dye test has traditionally been used to estimate the time of infection because the DT increases for approximately the first 8 weeks after infection; however, the dye test is expensive and difficult to perform and standardize, and only a limited number of laboratories perform it on a routine basis (22). The concept of measuring *Toxoplasma*-specific IgG antibody maturation as the immune response evolves was introduced for *T. gondii* by Hedman et al. (8), who showed that sera collected from infected patients up to 3 months after infection could be distinguished from those collected later.

The IgG avidity index is not standardized between laboratories, and no external quality assurance program includes the *Toxoplasma*-specific IgG avidity index. We do not know much about the variability and reproducibility of the index, but modeling has shown wide confidence intervals, and it is not known whether the avidity-maturation curve is linear over time (1). The maturation of the IgG response varies considerably between individuals. In a study by Lappalainen et al. (14), two seroconverting mothers already had an IgG avidity index above 20% at the time of diagnosis, but most patients had developed an IgG avidity index above 15% after 180 days (14). A study from France found an average IgG avidity index of 0.2 in pregnant women infected within 5 months (15).

The original method developed by Hedman et al. (8) used serial dilutions tested in an enzyme immunoassay with and without 6 M urea, but automated assays today calculate the IgG avidity index from two single measurements of a sample with and without urea. This process introduces uncertainty, although experiments with only two dilutions showed excellent agreement with IgG avidity measurements obtained with four serial dilutions (13). Testing of a single dilution with and without urea was further evaluated by Prince and Wilson (21), who showed that because the signal obtained in an enzyme immunoassay system is not linear, it makes a difference whether the *Toxoplasma*-specific IgG avidity index is calculated from optical densities or activities measured in international units of *Toxoplasma*-specific IgG antibodies per milliliter (21).

The avidity results found here showed that a persistent, low IgG avidity index is seen in many patients even more than 4 months after infection. Thus, a low IgG avidity index does not predict infection within the preceding 4 months, but a high IgG avidity index almost always excludes infection within the preceding 4 months in treated pregnant women and within 2 months in nontreated nonpregnant individuals.

It is necessary to know the date of infection to evaluate the performance of *Toxoplasma*-specific IgG avidity index assays. Up to half of patients with acute infections may show a low or borderline IgG avidity index 6 months after the infection (19, 24), in concordance with the results reported here. The LIAISON system was compared with the semiautomated VIDAS system for measuring the *Toxoplasma*-specific IgG avidity index; there was a good correlation between the results of the two systems, indicating that the persistence of low-level *Toxoplasma*-specific IgG antibodies is a problem inherent in measuring the *Toxoplasma*-specific IgG avidity index and is unrelated to the assay system.

We found one sample within the first 4 months after infection with an IgG avidity index just above 0.25. This finding is rare but has also been reported in other studies. For instance, the cutoff for the *Toxoplasma*-specific IgG avidity index in the VIDAS system was defined as 0.3 to ensure that all sera from acute infections had a low-avidity index (20). The same study showed that at least in pregnant women, a low IgG avidity index persisted for up to 9 months after infection, and all women were treated.

In a study of *T. gondii*-infected pregnant women identified prospectively through prenatal screening, Jenum et al. (11) found that 2 out of 73 women had an IgG avidity index above 0.2 before 20 weeks after infection, but many continued to have a low IgG avidity index even 1 year after infection. It is assumed that all women were treated during pregnancy.

The definition of a low IgG avidity index differs markedly between different studies, and one study found that patients infected within the preceding 3 months had an IgG avidity index below 0.45 (10). A comparison of the VIDAS and Labsystems IgG avidity index assays showed correlation coefficients of 0.6 for pregnant women and 0.88 for other patients (2), but the difference was not discussed further. Improvement of the IgG avidity assay with the Western blot technique was attempted and revealed differences in the maturation of specific IgG antibodies to different antigens (26).

The observation that *T. gondii*-specific IgG maturation is delayed in treated pregnant women compared to nontreated individuals was reported in one previous study, which found significantly delayed IgG maturation in treated individuals (25). Our finding that treatment may influence IgG maturation emphasizes the need for further studies to better clarify the avidity maturation process in pregnant women receiving therapy in comparison with nontreated individuals. Depending on the results of such studies, different cutoff values may have to be defined for treated and nontreated persons.

By use of *Toxoplasma*-specific IgG assays in combination with specific IgM antibodies, the diagnosis of *T. gondii* infection in early pregnancy has been improved significantly (23).

The LIAISON system is a valuable system for the exclusion of recently acquired infection with *T. gondii* (less than 4 months) in pregnant women and decreases significantly the necessity for follow-up testing.

ACKNOWLEDGMENT

This study was funded by DiaSorin S.p.A., Saluggia, Italy.

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