

LDBio-Toxo II Immunoglobulin G Western Blot Confirmatory Test for Anti-*Toxoplasma* Antibody Detection[∇]

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Tests commonly used for routine determination of anti-*Toxoplasma gondii* immunoglobulin G (IgG) antibodies show a high level of consistency. However, considerable variations between commercial screening tests are still observed in detecting antibodies present at low concentrations, leading to a number of discrepant and/or equivocal results. It is therefore important to use a reference test to confirm borderline results. In this study, we evaluated the use of a new qualitative test based on Western blot analysis—the LDBio-Toxo II IgG test—as a confirmatory test for at-risk patients. The study was performed retrospectively, using 569 serum samples with “low-positive” (2 to 32 international units) anti-*Toxoplasma* IgG levels from 375 patients. These samples were either sera collected during the routine screening of pregnant women, from patients with unrelated infections, or from immunocompromised patients or sequential sera taken from pregnant women with acquired *Toxoplasma* infection or from their newborns during follow-up. The LDBio-Toxo II IgG test was compared to several commercial tests commonly used for anti-*Toxoplasma* IgG screening. The Sabin-Feldman dye test was used as a reference test. In this study, the results of the LDBio-Toxo II IgG test appeared to be consistent with those of the dye test; the LDBio-Toxo II IgG test had a specificity of 100% and a sensitivity of 99.2%. Our findings suggest that the LDBio-Toxo II IgG test is a useful serological tool in cases in which the presence or absence of *Toxoplasma* antibodies needs to be reliably determined, for example, for the follow-up of pregnant women and their newborns or for subjects with immune deficiencies following human immunodeficiency virus infection, hematological malignancies, or transplantation.

Toxoplasmosis is the most frequent and widespread protozoal infection in humans. It is usually benign, but naturally acquired infections often lead to severe complications both in nonimmune pregnant women and in immunodeficient individuals. Additionally, life-threatening reactivation of previous infections is commonly observed in cases of severe immunodeficiency, such as with human immunodeficiency virus (HIV)-infected or organ transplant patients. Determination of the specific *Toxoplasma gondii* immune status in such patients is therefore essential for defining appropriate follow-up and prophylactic measures.

Immunoenzymatic tests are the most commonly used of a number of serological tests available for detecting anti-*Toxoplasma* antibodies. Regardless of the technique used, results are often equivocal when concentrations of specific immunoglobulin G (IgG) antibodies are close to the cutoff values. In these cases, a second confirmatory immunological test giving borderline results itself is not any more conclusive.

A qualitative test based on immunoblotting, the LDBio-Toxo II IgG test (herein referred to as the LDBio IgG test; LDBio Diagnostics, Lyon, France), which detects anti-*Toxoplasma gondii*-specific IgG, was recently developed to confirm serological test results for low titers of IgG antibodies. We evaluated the use of the LDBio IgG test for confirmation of current commercial anti-*Toxoplasma* IgG screening test results.

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MATERIALS AND METHODS

The LDBio IgG test is a qualitative immunoenzymatic test in which parasite antigens are separated by electrophoresis and then transferred by electroblotting to nitrocellulose strips. The kit includes the strips, a positive control, and all liquid reagents ready to use. The incubation times are standardized (60 min for the sample and anti-human IgG conjugate and 30 min for the nitroblue tetrazolium-BCIP [5-bromo-4-chloro-3-indolylphosphate] substrate). The resulting bands on the patient's strip are compared with the five bands on the positive-control strip, corresponding to 30, 31, 33, 40, and 45 kDa. A positive result is defined by the presence of at least three matching bands on the patient's strip, including the band at 30 kDa. The tests were performed in the Parasitology-Mycology laboratories of the la Timone and Saint Louis University hospitals. The Sabin-Feldman dye test (DT) was performed as the reference test at the Institut de Puériculture de Paris, France (P. Thulliez).

The LDBio IgG test was evaluated in a retrospective study using a total of 569 sera from 375 patients. Sera were stored frozen at -20°C before analysis. Groups of sera were selected as follows to examine the performance of the test in various diagnostic conditions.

Routine samples (group I). Group I comprised 200 sera originating from routinely tested pregnant women, including 102 negative and 98 “low-positive” (2 to 32 international units [IU]) samples, as determined by the DT. This group was designed for assessing the specificity and, in particular, the sensitivity of LDBio IgG in cases of low-positive sera. Sera were additionally tested with the Cobas Core Toxo IgG EIA II test (i.e., the Cobas IgG test; Roche Diagnostics, Meylan, France) and Vidas Toxo IgG II test (i.e., the Vidas IgG test; BioMérieux, Marnes La Coquette, France).

Sera from patients with unrelated infections (group II). For group II, we used 69 samples from 44 patients with viral infections (10 with HIV, 10 with hepatitis C virus, 2 with hepatitis A virus, 9 with hepatitis B virus, 5 with cytomegalovirus, 3 with varicella-zoster virus, and 5 with Epstein-Barr virus) and 25 patients with malarial infections to further assess the specificity of the LDBio IgG test in patients with unrelated infections. Test results were compared to those obtained with the Cobas IgG test. Sera with discordant results were also tested with the DT.

Sera with discordant or equivocal results (group III). For group III, we evaluated the use of the LDBio IgG test as a confirmatory test for 40 samples from 39 patients, mostly adults with immunosuppressive conditions, presenting discordant or equivocal results with routine tests (Platelia Toxo IgG [Bio-Rad,

TABLE 1. Comparison of three tests using routine serum samples with negative or low-positive results by DT (*n* = 200) (group I)

DT result (no. of sera)	No. of sera with indicated result						
	LDBio IgG test		Cobas IgG test		Vidas IgG test		
	Positive	Negative	Positive	Negative	Positive	Equivocal	Negative
Positive (98)	97	1	93	5	62	24	12
Negative (102)	0	102	0	102	0	1	101

Marnes-la-Coquette, France] and the Toxoscreen direct agglutination assay [Toxoscreen DA; BioMérieux]). Eighteen patients were infected with HIV, seven had hematological malignancies, four had aplastic anemia, two were stem-cell transplant donors, two were awaiting a renal transplant, one had vesicular dermatosis, and five were pregnant women. LDBio IgG confirmatory test results were assessed in comparison with results obtained with the DT.

We assessed the use of the LDBio IgG test for the follow-up of patients with congenital toxoplasmosis by using sequential sera from infected mothers and their newborns.

Infected mothers (group IV). Group IV comprised 101 sera from 17 mothers infected during pregnancy (between three and five sera per mother, including the last sample with negative results before seroconversion).

Newborns (group V and VI). Groups V and VI consisted of sera from infants born to mothers who had *Toxoplasma* seroconversion during pregnancy, comprising 74 sera from 20 noninfected newborns (group V) and 85 sera from 30 infected newborns (group VI).

The presence of congenital infection was determined through either antenatal detection of *T. gondii* DNA in amniotic fluid by using PCR—by detecting specific IgM/IgA in the infant’s serum at birth (immunosorbent agglutination assay IgM/IgA test; BioMérieux) and/or comparing mother and infant IgG/IgM Western blot profiles (*Toxoplasma* Western blotting IgG/IgM test; LDBio Diagnostics)—or detection of neosynthesized anti-*Toxoplasma* IgG antibodies in infant sera (Cobas IgG test) during the postnatal follow-up (between 5 and 12 months). The absence of congenital infection was defined by an undetectable level of IgG (Cobas IgG test) at 12 months in the absence of treatment.

Statistical analysis. Data were analyzed using McNemar’s chi-square test with Yates’ correction for small samples; *P* values of <0.05 were considered significant.

RESULTS

Routine samples (Tables 1 and 2). None of the 102 sera testing negative with the DT were found to be positive with the three other tests. Of the 98 DT low-positive samples, one serum sample (2 IU by the DT) had negative results by the LDBio IgG test, showing a unique band at 30 kDa (three of the five specific bands were required for a positive result). Five samples testing negative by the Cobas IgG test (<6 IU) tested positive with the DT (3.5 ± 1.5 IU [mean ±

TABLE 2. Performance parameters of three tests with routine serum samples with negative or low-positive results by DT (*n* = 200) (group I)^a

Confirmatory test	<i>P</i>	Sensitivity (%)	Specificity (%)	PPV (%) ^b	NPV (%) ^b
LDBio IgG test	1.00 (NS)	99.0	100	100	99.2
Cobas IgG test	0.63 (NS)	94.9	100	100	96.2
Vidas IgG test, with equivocal results considered:					
Positive	<0.0001	87.8	99.0	98.6	91.2
Negative	<0.0001	63.3	100	100	77.8

^a Performance values were calculated using the DT as the reference test.

^b NPVs and positive predictive values (PPVs) were calculated using the average prevalence of *Toxoplasma* infection in pregnant women in France in 2003 (43.8%) (4).

standard deviation]), and 12 samples testing negative by the Vidas IgG test (<4 IU) tested positive with the DT (3.3 ± 1.4 IU). Twenty-five samples (12.5% of the 200 sera tested) gave equivocal results with the Vidas IgG test (5 to 7 IU). One of these samples (7 IU by the Vidas IgG test) tested negative by the DT; the other samples tested positive (4.8 ± 1.3 IU by the DT). The decision to include the equivocal data as positive results substantially affected the calculated performance of the Vidas IgG test. According to the manufacturer’s instructions, sera with equivocal results should not be considered to have tested positive. The corresponding performance parameters of the test were thus a sensitivity of 63.3% and a negative predictive value (NPV) of 77.8%.

Sera from patients with unrelated infections (Table 3). There were four discrepant results for sera from patients with unrelated infections: 2 of the 25 Cobas IgG test-negative samples were positive by the LDBio IgG test, and 2 of the 44 Cobas IgG test-positive samples (7 and 8 IU/ml) were negative by the LDBio IgG test. DT results were consistent with the LDBio IgG test results in each of these four cases.

Discordant or equivocal results (Table 4). In group III, including 33 adults with immunosuppressive conditions, a 100% concordance rate was observed between the LDBio IgG test and the DT.

Seventeen samples which were negative by the Platelia IgG test (<6 IU) and either borderline or positive with the Toxoscreen DA tested positive with both the LDBio IgG test and the DT.

Of five sera that gave borderline results with the Platelia IgG test (6 to 9 IU) but were negative by the Toxoscreen DA, two were positive and three were negative by the LDBio IgG test and the DT.

The 18 remaining sera had a low-positive titer with the Platelia IgG test (28.5 ± 12.3 IU) and yielded a negative Toxoscreen DA result. Only one of these (17 IU) tested positive using the LDBio IgG test and the DT; the other 17 sera were confirmed negative by both tests.

Overall, for the 244 sera from groups I, II, and III tested with

TABLE 3. Evaluation of the LDBio IgG test using sera from patients with unrelated viral and protozoal infections (*n* = 69) (group II)

Cobas IgG test result (no. of sera)	No. of sera with indicated result			
	LDBio IgG test		DT	
	Positive	Negative	Positive	Negative
Positive (44)	42	2	ND ^a	2
Negative (25)	2	23	2	ND

^a ND, not done.

TABLE 4. Evaluation of the LDBio IgG test with sera with discordant Platelia IgG test and Toxoscreen DA results (*n* = 40) (group III)

Platelia IgG test result (no. of sera)	No. of sera with indicated result						
	Toxoscreen DA			LDBio IgG test		DT	
	Positive	Equivocal	Negative	Positive	Negative	Positive	Negative
Positive (18)	0	0	18	1	17	1	17
Equivocal (5)	0	0	5	2	3	2	3
Negative (17)	15	2	0	17	0	17	0

the LDBio IgG test and the DT as the reference test, the LDBio IgG test had a sensitivity of 99.2% and a specificity of 100%, giving an NPV of 99.4% and a positive predictive value of 100%.

Sequential sera from infected mothers (Table 5). In group IV, 10 of the 101 sequential serum samples (9.9%; *P* = 0.002) from 8 of 17 patients undergoing seroconversion (47%; *P* = 0.005) gave negative results with the Cobas IgG test and positive results with the LDBio IgG test. The Vidas Toxo IgM test and the immunosorbent agglutination assay IgM test detected high titers of specific anti-*T. gondii* IgM in these samples, consistent with a recently acquired infection. Both the Cobas IgG test and the LDBio IgG test detected specific anti-*T. gondii* IgG antibodies in further samples from the same patients two to three weeks later, confirming the seroconversion in these patients. Thus, newly synthesized IgG seems to be more likely to be detected at the beginning of seroconversion by using the LDBio IgG test than by using the Cobas IgG test (Fig. 1).

Sequential sera from newborns (Table 5). For 13 of the 74 sera of group V (17.6%; *P* < 0.0001), corresponding to 12 of 20 noninfected infants (60%; *P* < 0.0001), the results with the Cobas IgG test were negative, but the results with the LDBio IgG test were still positive (*P* < 0.0001). In three cases, a sample obtained later yielded negative results by both tests (Fig. 2).

In group VI, five samples from three congenitally infected children were negative by the Cobas IgG test but positive with the LDBio IgG test (*P* = 0.063 [not significant]). For one child treated with sulfadoxine-pyrimethamine (Fansidar), the Cobas IgG test yielded transitory negative results during the follow-

up, suggesting the absence of congenital infection, but this was not consistent with the results of the LDBio IgG test, which remained positive. The two other children were infected just before delivery. At the beginning of the follow-up, IgG antibodies were detected early in these two patients by using the LDBio IgG test, whereas the Cobas IgG test gave negative results.

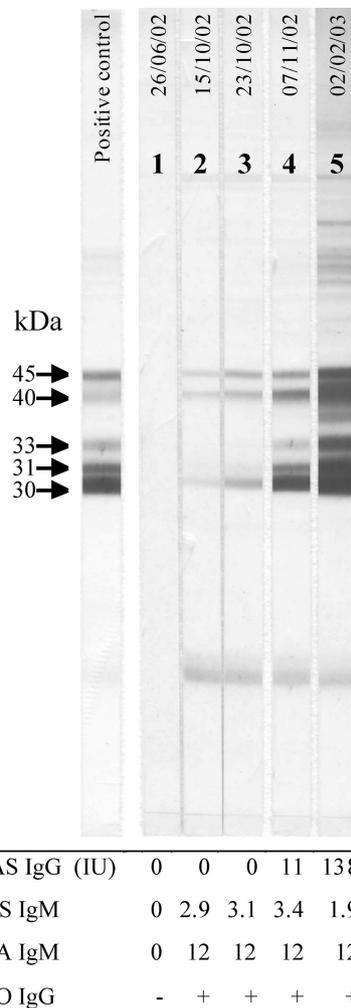


FIG. 1. Comparison of the results of the LDBio IgG test and the Cobas IgG test for sequential sera (at follow-up) from one patient who had undergone seroconversion. ISAGA, immunosorbent agglutination assay.

TABLE 5. Use of LDBio IgG test for monitoring congenital toxoplasmosis^a

Patient group (no. of sera) and Cobas IgG test result	No. of sera with indicated result by LDBio IgG test	
	Positive	Negative
Group IV (101)		
Positive	70	0
Negative	10	21
Group V (74)		
Positive	50	0
Negative	13	11
Group VI (85)		
Positive	80	0
Negative	5	0

^a *P* values for the differences between results of the two tests were 0.002 for group IV, <0.0001 for group V, and 0.063 (not significant) for group VI.

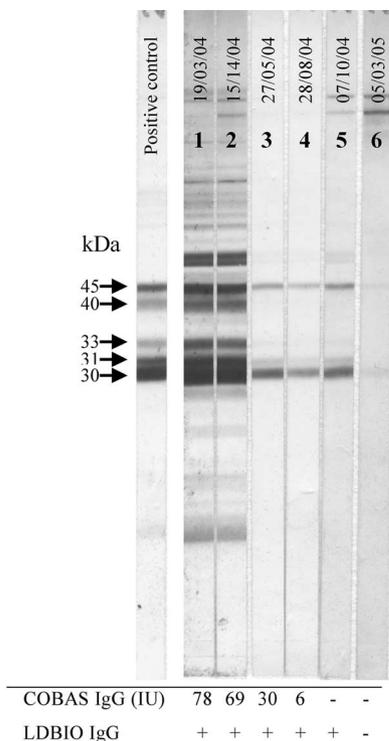


FIG. 2. Comparison of the results of the LDBio IgG test and the Cobas IgG test for sequential sera from one noninfected infant at follow-up.

DISCUSSION

Determining the specific immune status is essential for evaluating and preventing the risk of severe toxoplasmosis complications in susceptible individuals, such as nonimmune pregnant women or immunocompromised HIV-infected or transplant patients.

Sixty years after its introduction (23), the Sabin-Feldman DT is still used as the reference test for serological diagnosis of toxoplasmosis. This highly sensitive and specific test (1, 15, 21) remains the gold standard confirmatory test for discrepant and/or equivocal results. However, the DT is expensive and complex to maintain, perform, and standardize; thus, its use in laboratories is becoming less common (2, 20).

Consequently, a variety of homemade tests and commercial kits have been developed for detection of specific anti-Toxoplasma IgG antibodies; immunoenzymatic tests, which are well adapted to large-scale screening, are the most commonly used. Previous studies have evaluated the performance of these tests and have mostly found test results to be consistent among the various commercial kits (6, 18, 22). However, discrepancies between results from anti-Toxoplasma IgG tests are frequently observed for sera yielding borderline results with IgG concentrations close to the detection threshold; consequently, manufacturers often define a “gray zone,” corresponding to low antibody titers, in which results are considered inconclusive.

Our findings highlight the difficulties associated with interpreting serological results for low IgG values. Group I sera (IgG concentrations of <32 IU/ml by the DT) yielding discrepant results were those with the lowest IgG titers: using the

results for the samples giving DT IgG titers between 2 and 10 IU (n = 52) in this group, the sensitivity of the Cobas IgG test was 90.4% (five false-negative results), that of the Vidas IgG test was 58.6% (12 false-negative results, excluding 23 equivocal results), and that of the LDBio IgG test was 98.1% (one false-negative result).

These data highlight the benefit of using a confirmatory test to check samples with anti-Toxoplasma IgG titers close to the cutoff value. One such test is the DA, which has a good level of sensitivity. The main advantage of the DA is that it is commercially available (e.g., the Toxoscreen DA) and easy to perform in all laboratories. Nevertheless, the DA seems to be less reliable than the DT (14, 25) and, being a subjective analysis, can yield results that are equivocal or difficult to interpret for low-positive samples. This is illustrated by our results for group III, for which 3 of 23 Toxoscreen DA-negative samples and both samples with borderline results by the Toxoscreen DA were positive with the DT.

Thus, our findings suggest that the LDBio IgG test efficiently and reliably distinguishes between low-positive and negative sera, showing 100% specificity and 99.2% sensitivity compared to the DT.

Since 1978, the congenital toxoplasmosis prevention program in France has focused on the identification of seronegative pregnant women at risk of infection and their monthly serological follow-up during pregnancy. The results obtained from group I samples highlight the benefits of using a confirmatory test, such as the DT or the LDBio IgG test, to check samples with low levels of IgG in such patients (Tables 1 and 2). In this group, five mothers (2.5%) had false-negative Cobas IgG results, 12 mothers (6%) had false-negative Vidas IgG test results, and 36 mothers (18%) had equivocal Vidas IgG test results; serological follow-up of these patients could have been avoided with the use of the LDBio IgG test.

Toxoplasma serological screening programs for pregnant women require systematic testing for IgM antibodies in addition to IgG because of the delayed appearance of IgG following IgM in infected patients. However, some immunoenzymatic tests detecting anti-Toxoplasma IgM antibodies seem to have low positive predictive values and can yield discrepant results (7, 9, 17, 19). Therefore, seroconversion is often confirmed only once IgG is detected (5, 9, 13). For group IV (Table 5), IgG antibodies were detected by the LDBio IgG test in 10 sera with negative results by the Cobas IgG test (P = 0.002). In this series, the LDBio IgG test was able to confirm seroconversion in 8 of 17 infected mothers several weeks earlier than the Cobas IgG test (Fig. 1).

The absence of congenital infection in infants born to infected mothers can be ascertained only once consistently negative results are obtained from serological tests at a one-year follow-up, after the disappearance of maternal IgG antibodies. Samples from 12 of 20 group V (noninfected infants) yielded negative results with the Cobas IgG test at the end of the follow-up but remained positive with the LDBio IgG test (Table 5 and Fig. 2). The serum sample of one child in group VI (infected infants) who was treated with sulfadoxine-pyrimethamine yielded a transitory negative result with the Cobas IgG test. In such cases, the use of the Cobas IgG test may lead to the premature or wrong conclusion that congenital infection is absent. This emphasizes the importance of using a

sensitive confirmatory test for the serological follow-up of newborns at risk of congenital infection. However, the LDBio IgG test cannot distinguish fetal IgG from maternal transmitted IgG. Therefore, this test cannot be used for the diagnosis of congenital toxoplasmosis at birth.

Severe complications following *Toxoplasma* infection, associated with suppression of the host's immunity, are often observed in patients, particularly in HIV-infected and transplant patients. Such patients can develop toxoplasmosis by natural transmission or following two major events, namely, allograft in solid-organ-transplant recipients and recrudescence of a previous infection following cyst disruption, frequently observed in HIV-infected patients or stem-cell transplant recipients. Therefore, serological testing is essential for evaluating the toxoplasmosis risk for these patients (6, 8, 16, 24, 26). Posttransplant toxoplasmosis has been reported in mismatched solid-organ-transplant recipients (positive donor/negative recipient), mostly in those receiving heart or heart-lung transplants. Stem-cell allogeneic grafts carry a major risk of toxoplasmosis reactivation in seropositive recipients, especially if the donor is negative (3). Likewise, in HIV-infected patients and other immunocompromised patients, there is a high risk of reactivation for seropositive individuals experiencing a profound alteration of the immune system. In all cases, the accurate determination of the immune status of the patient or the donor/recipient in terms of *T. gondii* antibodies is essential; thus, the choice of the serological tests used is crucial (3, 5, 10, 11, 12). For instance, 35 samples of group III sera (those with discordant results; in total, 40 samples) were from immunodeficient patients; for this series of patients, the LDBio IgG test gave unequivocal results, revealing 17 false-positive results obtained with the Platelia IgG test, 17 false-negative results obtained with the Platelia IgG test, and 3 false-negative results obtained with the Toxoscreen DA.

In conclusion, the LDBio IgG test represents an accurate and useful serological tool in situations in which serological tests are needed to determine the presence or absence of *Toxoplasma* antibodies, such as in pregnant women and their newborns or in subjects with immune deficiencies following HIV infection, hematological malignancy, or transplantation. Although not designed for high-throughput screening, the LDBio IgG test can be used as an easy and very reliable confirmatory test in laboratories where the DT cannot be implemented, when routine tests show discordant, equivocal, or borderline results.

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