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Screening for Active Toxoplasmosis in Patients by DNA Hybridization with the ABGTg7 Probe in Blood Samples

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We report the potential use of a specific *Toxoplasma gondii* DNA probe (ABGTg7). We applied a dot blot hybridization assay to blood samples for the diagnosis of cerebral toxoplasmosis (CT), acute toxoplasmic lymphadenopathy (ATL), and disseminated toxoplasmosis in transplant recipients (TRs). We studied a total of 84 individuals: 38 patients and 46 controls. We found positive hybridization signals for 12 (66.7%) of 18 patients with confirmed CT, 9 (52.9%) of 17 patients with ATL, and 2 (66.7%) of 3 TRs. PCR assays were performed in parallel for patients with ATL, resulting in *T. gondii* DNA detection for 10 patients (58.8%). A comparative study between dot blot and PCR assays performed with the blood of mice that had been experimentally infected with tachyzoites gave similar results: 60 and 70% positive results, respectively. Finally, the sum of positive values obtained by both DNA tests (dot blot assay plus PCR) increased the rate of positivity for ATL patients to 76.4%. These results demonstrate that the *T. gondii* ABGTg7 repetitive DNA element is an additional useful resource for diagnosing *Toxoplasma* parasitemia in patients with CT and ATL and in TRs. Thus, our ABGTg7-based dot blot test may lead to an improvement in *T. gondii* detection methods in patients with acute toxoplasmosis.

Human infection with *Toxoplasma gondii* is normally not symptomatic. However, in some individuals such as immunocompromised patients (transplant recipients and AIDS patients) toxoplasmosis can be life-threatening. The incidence of toxoplasmic encephalitis in patients with AIDS is growing, reaching alarming proportions (23, 24, 28). Due to the immunological conditions of these patients, serological diagnosis is often ambiguous because of the absence of rising anti-*Toxoplasma* immunoglobulin (IgG) titers and high IgM titers (24, 27). Direct detection procedures such as microscopic examination, immune histology, or cell culture are either insensitive or time-consuming (24). In patients with human immunodeficiency virus infection, diagnosis of cerebral toxoplasmosis (CT) relies on a compatible clinical presentation, serological evidence of exposure to *T. gondii*, and characteristic computed tomographic scan. The diagnosis is confirmed by patient response to specific treatment. Thus, Porter and Sande (27) considered a patient to have toxoplasmosis if there was histological evidence of central nervous system infection, in addition to brain lesions. The difficulty in establishing the diagnosis of CT without performing a brain biopsy encouraged us to search out a noninvasive method for the direct diagnosis of active toxoplasmosis in blood and/or cerebrospinal fluid (CSF). Moreover, the presence of *T. gondii* tachyzoites in biological fluids indicates active infection. In recent years, molecular biology techniques have successfully been applied to the detection of parasite DNA in clinical samples. Until now, five *Toxoplasma* DNA sequences have been chosen for PCR tests (30). One of these DNA fragments is a repetitive (35-fold) sequence named the B1 gene (5). Guy and Joynson (15) obtained positive PCR results for 53% of 17 patients using B1 primer pairs with the blood of patients with active toxoplasmic lymphadenopathy (ATL) when the blood samples were collected within the first 5 weeks of illness. Thus, B1 primer pairs were also used for the detection of parasitemia in AIDS patients with CT or extracerebral toxoplasmosis (11, 19). Furthermore, parasitemia was detected in 12 of 32 blood samples (37%) from rabbits experimentally infected with *T. gondii* tachyzoites by PCR amplification of B1 genes, while by mouse inoculation, parasitemia was detected in 20 of 32 blood samples (62%) (16). Mouse inoculation requires the use of living animals and has a long turnaround time. Therefore, most of the direct *T. gondii* detection strategies are based on PCR techniques (30).

Previously, we isolated three repetitive DNA elements from the *T. gondii* genome (1, 3). We had described the application of one of them (ABGTg4) for the detection of parasite DNA by a dot blot assay in CSF (2) and in a blood sample from a patient with a rare case of toxoplasmosis (12). In an effort to improve the reliability and simplicity of DNA-based *T. gondii* detection methods, we recently isolated a new repetitive DNA element of the ABGTg4 family. It was named ABGTg7, and it contains the 427 bp of the ABGTg4 sequence plus an additional 309 bases. Moreover, the ABGTg7 element includes two repetitive units of the ABGTg4 repetitive DNA family. Here, we show that the ABGTg7 element can be used as a sensitive tool for the diagnosis of the presence of parasite DNA by dot blot hybridization. Comparing our newly developed ABGTg7 dot blot assay to the commonly applied B1 primer-based PCR test, we demonstrated that our test may be as efficient as PCR analysis, leading to an improvement in already existing *T. gondii* detection assays.

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MATERIALS AND METHODS

Patients whose blood was analyzed by dot blot assay. We studied a total of 84 individuals (see Table 1). The patients were analyzed as follows: clinical presentation, serology for toxoplasmosis, and computed tomography image. We also

performed serological studies for *Cryptococcus*, *Mycobacterium*, and *Trypanosoma*, cultures for fungi, and specific tests for herpesvirus, cytomegalovirus, and syphilis. CD4 lymphocyte counts were also occasionally performed. For all AIDS patients with a diagnosis of active toxoplasmosis, CD4-cell counts were between 20 and 300/mm^3. All groups were analyzed in a blinded manner.

(i) Individuals with encephalitis. The group of individuals with encephalitis consisted of 29 patients. Eighteen of them were confirmed to have CT, and 11 of them were confirmed to have encephalitis caused by other organisms and were classified as patients with nontoxoplasmic encephalitis. Definitive active CT was established either when the patient recovered clinically and tomographically after specific treatment with pyrimethamine and sulfadiazine or by histological and immunohistological examination of biopsy or autopsy samples. Samples were obtained before any specific therapy was applied.

(ii) Individuals with ATL. The group of individuals with ATLs consisted of 17 patients. Acute toxoplasmic infection was defined by the following criteria: (i) symptoms clinically compatible with acute toxoplasmic infection such as cervical lymphoadenopathy, fever, and myalgia and (ii) IgM and IgG seroconversion from nondetectable anti-*T. gondii* antibodies to different levels of positive reactions. The serological status of the patient was determined by performing an indirect immunofluorescence (IIF) test for either IgG or IgM antibodies, and specific IgM antibodies against *T. gondii* antigens were also identified by immunosorbent agglutination assay (ISAGA). Since the individuals were immunocompetent, no therapy was administered.

(iii) Transplant recipients. We studied six patients who underwent either a kidney or a heart transplant. Three of them had confirmed toxoplasmosis, as judged by patient recovery after specific treatment with pyrimethamine and sulfadiazine or by histological and immunohistological examination of biopsy samples.

(iv) Individuals with chronic toxoplasmic infection. Samples from subjects with positive IIF test results and negative ISAGA and IIF IgM test results were placed in a group considered to have chronic toxoplasmosis (19 patients).

(v) Seronegative individuals. Thirteen immunocompetent individuals who did not show antibodies against *Toxoplasma* antigens were also studied.

Microorganisms. *T. gondii* tachyzoites of the RH strain were grown in the peritoneal cavities of CF1 mice.

Plasmodium falciparum, *Plasmodium vivax*, *Echinococcus granulosus*, and *Pneumocystis carinii* DNA samples were provided by Eduardo Guarnera, Departamento Parasitología, Instituto Nacional de Microbiología Dr. Carlos G. Malbran. *Mycobacterium tuberculosis* and *Mycobacterium avium* were kindly provided by Lucia Barrera, Departamento Micobacterias, Instituto Nacional de Microbiología Dr. Carlos G. Malbran. *Candida albicans*, *Cryptococcus neoformans*, *Nocardia asteroides*, and *Nocardia brasiliensis* were kindly provided by Graciela Davel, Departamento Micologia, Instituto Nacional de Microbiología Dr. Carlos G. Malbran.

Animal model. Five male and five female mice of the CF1 strain (age, 2 months) were inoculated intraperitoneally with 1,000 *T. gondii* tachyzoites of the RH strain. On the fifth day postinoculation, peripheral blood samples were extracted and placed in 5 mM EDTA. Two mice were kept uninfected for use as negative controls.

Preparation of DNA samples. Five milliliters of blood was collected from each individual in EDTA-containing tubes. Later, two volumes of 150 mM NaCl were added to 5 aliquots of 300 μl each (in total, 1.5 ml of blood per individual). They were frozen and thawed once, centrifuged for 1 min in a microcentrifuge, and washed twice with TE (Tris-HCl [10 mM], EDTA [1 mM] [pH 8]). The pellet was resuspended in 150 μl of TES buffer (Tris HCl [10 mM], EDTA [150 mM], NaCl [150 mM] [pH 7.4]) per 300 μl of blood plus 1% sodium dodecyl sulfate (SDS) and 200 μg of proteinase K per ml. The samples were incubated for 2 h at 56°C. The DNA was extracted with equal volumes of phenol, chloroform-isoamyl alcohol (24:1), and ether and precipitated with 2 volumes of ethanol. Finally, the DNA was centrifuged and resuspended in 500 μl of TE. The DNA concentration was determined at 260 nm by spectrophotometry in a Shimadzu PR-1 spectrophotometer as described previously (25). If CSF was available, the DNA was extracted in the same way described above for blood, except that the first two steps were not performed with CSF.

DNA test by dot blot assay. Ten micrograms of genomic DNA was spotted onto nitrocellulose filters (Hybond ECL Western; Amersham) by applying vacuum conditions (GS Gene Prep Manifold System; Bio-Rad), and the filters were processed as described previously (3). Hybridizations were performed in 6× SSC (20× SSC is 0.3 M sodium Citrate plus 3 M NaCl), 0.1% SDS, and 1% blocking reagent from the DIG DNA labeling kit (Boehringer, Mannheim, Germany) at 60°C. Probe ABGTg7 is a *T. gondii* repetitive double-stranded DNA that was labeled with digoxigenin-dUTP by using a random primer labeling kit according to the instructions of the manufacturer (DIG labeling kit system; Boehringer). Probe ABGTg7 was used at concentrations of 25 ng/ml. Washes were performed under high-stringency conditions (0.1× SSC and 1% SDS; 65°C). Each dot blot hybridization assay was performed with *T. gondii* tachyzoite DNA as positive controls and 10 μg of DNA from a noninfected mouse as a negative control. Signals were classified as follows: positive when their intensities were equal to or higher than that for 5,000 parasites and negative when their intensities were lower than that for 5,000 parasites. This cutoff was determined from experiments that had been performed with negative DNA controls that hybridized with the ABGTg7 probe.

PCR. The target DNA for PCR amplification was the published sequence of the B1 gene from *T. gondii* RH (5). Amplification was performed in a Sontec Científica Argentina thermal cycler with 2.5 U of *Taq* DNA polymerase (BRL) in a final reaction volume of 50 μl with 1× BRL buffer (10 mM Tris-HCl [pH 8.4], 50 mM KCl) plus 2.5 mM $MgCl_2$, 0.2 mM (each) deoxynucleotide triphosphate, 1 μmol (per liter) each of the oligonucleotides 1 (5′-GGAACTGCATC CGTTCATGAG-3′; positions 694 to 714) and 4 (5′-TCTTTAAAGCGTTCGT GGTC-3′; positions 887 to 868) (5), and 1 μg of the DNA to be amplified. The samples were overlaid with mineral oil. Control reactions without any DNA were always carried out in parallel. In addition, each amplification with a patient sample was carried out in parallel with 50 pg of *T. gondii* DNA in order to test for the presence of possible *Taq* DNA polymerase inhibitors. Samples were amplified for 40 cycles, as follows: an initial cycle of 5 min of denaturation at 94°C, 30 s of annealing at 55°C, and 60 s of extension at 72°C. The remaining cycles consisted of 45 s of denaturation at 94°C, 60 s at 55°C, and 90 s at 72°C; and cycle 40 was followed by an additional 10 min of incubation at 72°C. Strict methods for avoiding contamination were used, such us performing the different steps in three separate rooms, using a separate set of pipettes only for preparing the reaction mixture, and using aerosol-resistant tips. Samples were assayed at least twice. The PCR products were run on a 1.5% agarose gel, and the results were visualized by ethidium bromide staining. Dot blot hybridization was used to determine the specificity of the PCR product. To achieve this, hybridization with internal oligonucleotide 3 (5′-GGCGACCAATCTGCGAATACACC-3′; positions 831 to 853) (5), with the 5′ end biotinylated, was carried out. Briefly, samples were boiled for 10 min and were spotted onto nitrocellulose membranes with a dot spot apparatus (GS Gene Prep Manifold System; Bio-Rad). The DNA was covalently bound to the nitrocellulose membranes by baking the membranes at 80°C for 2 h. The filters were prehybridized in 6× SSC–5× Denhardt solution–0.1% SDS–250 μg of salmon sperm DNA per ml at 43°C for 2 h. Hybridizations were performed at 43°C for 18 h in 6× SSC–0.1% SDS–1× Denhardt solution–35 pg of the probe per ml. After hybridization, the membrane filters were washed twice in 6× SSC–0.1% SDS at 43°C for 15 min each time. Finally, the membrane filters were developed with a nonradioactive nucleic acid detection system (BluGENE; BRL) according to the instructions of the manufacturer.

Data analysis of dot blot and PCR assays. Blood samples from the 17 patients in the ATL group and 10 mice experimentally infected with *T. gondii* tachyzoites were assayed in parallel by dot blot and PCR tests (see Table 2). Each assay was repeated twice with different DNAs extracted from the same blood sample. After interassay comparisons, those samples that gave different results were assayed at least two more times in order to confirm the previous results. Thus, some samples were arbitrarily chosen and repeatedly assayed by dot blot and PCR assays to establish the reproducibility of the assays.

Statistical methods. Confidence intervals for the percentage of positive results were obtained by using a binomial distribution.

Nucleotide sequence accession number. The nucleotide sequence accession number for probe ABGTg7 is X74557.

RESULTS

***T. gondii* DNA detection by dot blot assay of blood from patients with active toxoplasmosis.** We studied 38 patients with acute toxoplasmosis infection by the dot blot hybridization assay using repetitive DNA probe ABGTg7 (Table 1, group A). Our results revealed that 60.5% of blood samples from these patients gave positive signals by the dot blot assay (Table 1, group A).

In order to compare the diagnostic value of the dot blot assay with blood from patients with different forms of acute toxoplasmic infection, we listed the data separately in Table 1. *T. gondii* DNA was detected in 12 of 18 patients with active CT (66.7%), 17 of 26 patients with ATL (52.9%), and 3 of 5 transplant recipients (66.7%) (Table 1, group A). Interestingly, we obtained the same percentage of positive signals by the dot blot assay when we used CSF samples from individuals with CT (data not shown).

The dot blot assay with the ABGTg7 probe was demonstrated to be quite specific, since no false-positive results have been observed. As shown in Table 1 (group B), no positive signals were detected for the different groups with nonactive toxoplasmic infection.

Furthermore, no cross-hybridization was observed with 400 to 500 ng of DNA from organisms clinically related to *T. gondii* such as *P. carinii*, *C. neoformans*, *M. tuberculosis*, *M. avium*, *N. asteroides*, and *N. brasiliensis* or from others pathogens as *P.*

TABLE 1. *T. gondii* DNA detection by dot blot assay blood from patients with acute toxoplasmosis and controls

Patient group[a]	No. of samples tested	No. (%) positive
Group A		
Toxoplasmic encephalitis patients	18	12 (66.7)[b]
ATL patients	17	9 (52.9)[c]
Kidney and heart transplant recipients with toxoplasmosis	3	2 (66.7)
Total	38	23 (60.5)[d]
Group B		
Nontoxoplasmic encephalitis patients	11	0 (0.0)
Chronic toxoplasmosis patients	19	0 (0.0)
Kidney and heart transplant recipients without toxoplasmosis	3	0 (0.0)
Seronegative individuals	13	0 (0.0)
Total	46	0 (0.0)

[a] Group A, patients with confirmed active toxoplasmosis; group B, patients without toxoplasmosis or with chronic toxoplasmosis.
[b] The 95% CI was 45.7 to 87.7%.
[c] The 95% CI was 29.9 to 75.9%.
[d] The 95% CI was 45.5 to 75.5%.

falciparum, *P. vivax*, *E. granulosus*, and *C. albicans* (data not shown).

Comparative study of dot blot assay versus PCR for *T. gondii* DNA detection in patients with ATL. In order to assess the correlation between the dot blot hybridization assay with the ABGTg7 probe and a PCR test that is frequently used internationally (we regularly use one test based on the B1 gene [5]), we performed a parallel study using both techniques. Figure 1 gives an example of the results obtained by the dot blot assay and the PCR test with blood samples from nine ATL patients. The result of the dot blot assay was considered to be positive when the signal intensity was equal to or higher than that for 5,000 parasites. On the basis of the intensities of the spots, patients 1, 3, 4, 5, and 8 were positive (Fig. 1A). On the other hand, patients 2, 3, 5, and 8 were apparently positive by PCR (this is based on the obvious bands on the gel [Fig. 1B]). PCR results were confirmed by dot blot hybridization with an internal biotinylated probe (data not shown).

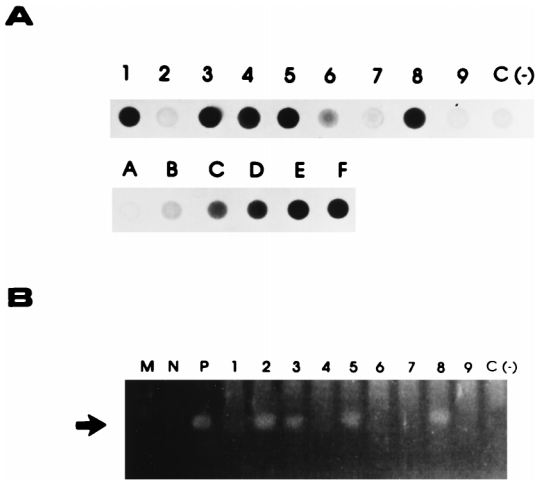

FIG. 1. Detection of *T. gondii* DNA in blood of ATL patients (dots 1 to 9) and a negative control [dot C(−)]. (A) Ten micrograms of blood from patients was spotted onto nitrocellulose filters and hybridized with a digoxogenin-dUTP-labeled ABGTg7 probe. Different quantities of *T. gondii* genomic DNA were hybridized with the ABGTg7 probe representing 0 (dot A), 1,000 (dot B), 5,000 (dot C), 10,000 (dot D), 20,000 (dot E), and 50,000 (dot F) tachyzoites, plus 5 μg of negative DNA. (B) The amplification products of the patient blood samples were analyzed after agarose gel electrophoresis and ethidium bromide staining. The markers used were ϕX174-*Hae*III DNA (lane M). The arrow indicates the size of the expected amplification product. Lane N, negative control consisting of PCR amplification without DNA; lane P, positive control, consisting of PCR amplification of 1 ng of *T. gondii* DNA.

TABLE 2. Comparative study of dot blot versus PCR for patients with ATL and experimentally infected mice

Study group and PCR result	No. of samples with the following dot blot assay result:		
	Positive	Negative	Total
Patients with ATL			
Positive	6	4	10[a]
Negative	3	4	7
Total	9[b]	8	17
Experimentally infected mice			
Positive	6	1	7[c]
Negative	0	3	3
Total	6[d]	4	10

[a] Total, 58.8% (95% CI = 35.8 to 85.8%).
[b] Total, 52.9% (95% CI = 29.9 to 75.9%).
[c] Total, 70.0% (95% CI = 42.0 to 98.0%).
[d] Total, 60.0% (95% CI = 30.0 to 90.0%).

In total, 9 of 17 samples (52.9%) gave positive signals by the dot blot assay and 10 of 17 samples (58.8%) produced a PCR amplification product of the correct size (Table 2). However, for 7 of 17 samples (41.1%), we observed different results between the two DNA detection systems. Three of 17 samples gave positive signals by the dot blot assay but were negative by PCR (17.6%). In contrast, four samples (25.5%) were positive by PCR but negative by the dot blot assay (Table 2). Total or partial inhibition was observed only for these PCR-negative, dot blot assay-positive samples.

Comparative study of dot blot assay versus PCR for *T. gondii* DNA detection in experimentally infected mice. In order to analyze in more detail the comparative study of the dot blot assay and PCR with blood samples for the detection of active *Toxoplasma* dissemination, we developed an experimental *T. gondii* infection in CF1 mice. In this assay, parasite DNA was detected by dot blot assay in the blood of 60% of 10 experimentally infected mice and in the blood of 70% of the mice by PCR (Table 2). Again, 1 of 10 samples gave different results by the two techniques (Table 2).

Complementation of dot blot assay and PCR for the diagnosis of active toxoplasmosis. A total of 35.3% (95% confidence interval [95% CI] = 16.6 to 54.0%) of 17 samples from patients with ATL gave positive values by the dot blot assay and the PCR test. The sum of positive values for both DNA detection systems increased the predictive diagnostic value to 76.4% (95% CI = 56.4 to 96.4%) (Table 2).

DISCUSSION

In this work, we have evaluated the diagnostic value of a dot blot assay with the recently isolated ABGTg7 probe. We investigated biological fluid samples from patients with active CT and ATL and transplant recipients. ABGTg7 is a DNA sequence that has two units of the repetitive DNA element of the ABGTg4 family (unpublished data). ABGTg4 is a high-copy-number repetitive *T. gondii* DNA sequence (1) which enabled us to detect as little as 800 parasites in 300 μl of blood by dot

blot hybridization assay, and no cross-hybridization was detected with *Eimeria*, *Leishmania*, *Trypanosoma*, human, or mouse DNA (3). Previously, we showed the usefulness of ABGTg4 as a probe for the detection of *T. gondii* DNA in the CSF of patients with CT and in the blood of a patient with a rare case of toxoplasmosis (2, 12). Using such a methodology, we extended the study in order to evaluate the application of this simple and rapid test to blood and CSF samples from patients with CT and blood of patients with ATL and transplant recipients. *T. gondii* DNA detection by dot blot assay of blood and CSF samples from patients with CT and blood samples of from transplant recipients led to similar results (66.7%). Parasitemia could be detected in 52.9% of 17 blood samples from patients with ATL. These results were obtained independently with a high degree of reproducibility.

The fact that the percentage of positive signals was higher in samples from patients with CT and transplant recipients than in samples from patients with ATL argues that parasite dissemination could be a feature of toxoplasmic reactivation in immunodeficient individuals. However, Khalifa et al. (19) detected by PCR little parasitemia in the blood of AIDS patients with CT. They concluded that in patients with CT, DNA detection by PCR could be useful only for patients with severe cerebral infection or dissemination of this disease. The high percentage of positive values found by the dot blot assay could be explained by the fact that we performed the assay with blood from AIDS patients who presented with severe manifestations of CT. Despite the advanced neurological symptoms of the patients, we consider the dot blot assay to be sufficiently quick (2 to 3 days) to apply a specific and successful therapy.

Accurate and rapid diagnosis of active *Toxoplasma* infection in patients with CT or congenital toxoplasmosis or in transplant recipients is a prerequisite for patient management. To achieve this aim, some investigators have carried out PCRs with low-copy-number target genes of the *T. gondii* genome (4, 5, 10, 11, 13–22, 26, 29, 30). The PCR test showed a high sensitivity, detecting 1 to 10 *T. gondii* tachyzoites when the DNA of purified parasites was used. Thus, the PCR assay had been described as a powerful method for the diagnosis of active toxoplasmosis in humans. Using blood samples isolated from subjects with ATL, Guy and Joynson (15) detected *T. gondii* parasitemia by PCR with B1 gene primer pairs in 53% of 17 ATL patients when the initial blood samples were collected within the first 5 weeks of illness. Dupuoy-Camet et al. (10) obtained positive PCR results for blood samples from 9 of 14 patients with presumptive CT (68%) using P30 gene-derived primer pairs. Dupon et al. (9) obtained positive PCR results for 10 of 13 CT patients (76.9%) using both CSF and blood samples and a ribosomal DNA sequence, but their data obtained in tests with blood showed only a 40% detection ability. However, the use of the PCR test often leads to contradictory results for the diagnosis of *Toxoplasma* infection. Ho-Yen et al. (18) did not find any positive *T. gondii* DNA amplifications for 13 patients with ATL. Lavrard et al. (20) compared the PCR test with cell culture and Giemsa staining of bronchoalveolar lavage samples from patients with pulmonary toxoplasmosis. They could not observe any difference among the assays. Finally, the investigators commented that they assayed 10 blood samples from patients with CT by PCR test but did not find any positive results.

Our results by PCR were consistent with those found by Guy and Joynson (15) when we used blood samples from ATL patients (58.8%). When we compare the diagnostic value of the dot blot hybridization assay and the PCR test, we found similar results (52.9 versus 58.8%, respectively), with a slightly superior diagnostic value for the PCR assay with samples from patients with ATL. In the experimentally infected mouse model, the dot blot assay detected *T. gondii* DNA in 60% of the mice tested, while the PCR test detected *T. gondii* DNA in 70% of the mice tested. For some samples, only one of the techniques, either the dot blot assay or PCR, is capable of detecting *T. gondii* DNA. A negative dot blot test result with a positive PCR test result may reflect either that the PCR result was false positive or that PCR is more sensitive for detecting lower levels of parasites. Since we did not observe false-positive PCRs for our negative control group, the latter explanation is the most likely. Positive dot blot assay results and negative PCR results could be explained by the total or partial inhibition of amplification reactions due to the presence of inhibitors in the samples, as was shown when DNA samples were spiked with 50 pg of *T. gondii* DNA (equivalent to approximately 500 parasites). At any rate, we point out that the use of both DNA methods allowed us to increase the rate of detection of *Toxoplasma* DNA to 76.4% for ATL patients.

ABGTg7 is a highly polymorphic tandemly repeated *T. gondii* DNA sequence with a unit length that ranges between 330 and 350 bp (unpublished data). The ABGTg7 repetitive DNA family was shown to be highly specific for *T. gondii* parasites since it did not cross-hybridize with DNAs from several organisms. Sequences of the same family are $TGR1_A$, $TGR1_E$, TGR2, and TGR4 (6). $TGR1_E$, a DNA sequence highly homologous with the ABGTg7 sequence (82%), detected DNA from 15 either virulent or avirulent *T. gondii* strains (8). Cristina et al. (7) used primers designed from the $TGR1_E$ sequence in a PCR assay in bronchoalveolar lavage fluid samples from AIDS patients with pulmonary toxoplasmosis, with successful results. However, until now we failed to complement the dot blot hybridization assay results by PCR with primers derived from the ABGTg7 sequence. Therefore, we are doing an exhaustive analysis of the ABGTg7 family of sequences before we design new primer pairs for PCR.

In conclusion, we showed that the dot blot assay with the nonradioactive ABGTg7 probe meets the speed, simplicity, sensitivity, specificity, and reproducibility requirements for a simple direct diagnostic test for active human toxoplasmosis in patients with CT and ATL or transplant recipients, especially when blood samples are used. The method for obtaining blood is noninvasive, and hence, the assay material is easy to obtain. In addition, as yet the dot blot assay has not shown any false-positive results for any of the samples that we studied. We propose that the dot blot assay with the ABGTg7 probe may be an alternative for the detection of *T. gondii* DNA in biological fluids. Moreover, the dot blot assay and PCR test showed independent results in the samples that we studied. In light of these features, the combination of dot blot assay with the PCR test could be an interesting and successful complementation to improving the detection of *T. gondii* DNA, at least in blood samples.

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REFERENCES

1. **Angel, S. O., J. C. Blanco, E. Maero, V. Pszenny, and J. C. Garberi.** 1991. Repetitive DNA sequences of *Toxoplasma gondii* for development of diagnostic probes. Mem. Inst. Oswaldo Cruz-Rio **86:**483–484.
2. **Angel, S. O., E. Maero, J. C. Blanco, V. Pszenny, C. Zala, R. Gonzalez, H.**

Perlmulter, and J. C. Garberi. 1992. Early diagnosis of toxoplasmic encephalitis in AIDS patients by dot-blot hybridization analysis. J. Clin. Microbiol. **30:**3286–3287.
3. **Blanco, J. C., S. O. Angel, E. Maero, V. Pszenny, P. Serpente, and J. C. Garberi.** 1992. Cloning of repetitive DNA sequences from *Toxoplasma gondii* and their usefulness for parasite detection. Am. J. Trop. Med. Hyg. **46:**350–357.
4. **Bretagne, S., J. M. Costa, J. Fleury-Feith, F. Poron, M. L. Dubreuil-Lemaire, and M. Vidaud.** 1995. Quantitative competitive PCR with bronchoalveolar lavage fluid for diagnosis of toxoplasmosis in AIDS patients. J. Clin. Microbiol. **33:**1662–1664.
5. **Burg, J. L., C. M. Grover, P. Pouletty, and J. C. Boothroyd.** 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by the polymerase chain reaction. J. Clin. Microbiol. **27:**1787–1792.
6. **Cristina, N., M. F. Liaud, F. Santoro, B. Oury, and P. Ambroise-Thomas.** 1991. A family of repeated DNA sequences in *Toxoplasma gondii*: cloning, sequence analysis, and use in strain characterization. Exp. Parasitol. **73:**73–81.
7. **Cristina, N., F. Derouin, H. Pelloux, R. Pierce, M. F. Delauwn, and P. Ambroise-Thomas.** 1992. Detection de *Toxoplasma gondii* chez des patients sidéens par la technique de "polymerase chain reaction" (PCR), à l'aide de la séquence répétée $TGR1_E$. Pathol. Biol. **40:**52–55.
8. **Cristina, N., M. L. Dardé, C. Boudin, G. Tavernier, M. Pedestre, and P. Ambroise-Thomas.** 1995. A DNA fingerprinting method for individual characterization of *Toxoplasma gondii* strains: combination with isoenzymatic characters for determination of linkage groups. Parasitol. Res. **81:**32–37.
9. **Dupon, M., J. Cazenave, J. Pellegrin, J. Ragnaud, A. Cheyrou, I. Fischer, B. Leng, and J. Lacut.** 1995. Detection of *Toxoplasma gondii* by PCR and tissue culture in cerebrospinal fluid and blood of human immunodeficiency virus-seropositive patients. J. Clin. Microbiol. **33:**2421–2426.
10. **Dupuoy-Camet, J., S. Lavareda de Souza, C. Maslo, A. Paugam, A. G. Saimot, R. Benarous, C. Tourte-Schaefer, and F. Derouin.** 1993. Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. J. Clin. Microbiol. **31:**1866–1869.
11. **Filice, G. A., J. A. Hitt, C. D. Mitchell, M. Blackstad, and S. W. Sorensen.** 1993. Diagnosis of *Toxoplasma* parasitemia in patients with AIDS by gene detection after amplification with polymerase chain reaction. J. Clin. Microbiol. **31:**2327–2331.
12. **Garberi, J. C., S. O. Angel, P. Paulin, V. Pszenny, and L. Romano.** 1994. Use of a monoclonal antibody and a DNA probe for diagnosing acute toxoplasmosis in ambiguous cases. J. Clin. Pathol. **47:**853–854.
13. **Gross, U., A. Roggenkamp, K. Janitschke, and J. Heesemann.** 1992. Improved sensitivity of the polymerase chain reaction for detection of *Toxoplasma gondii* in biological and human clinical specimens. Eur. J. Clin. Microbiol. Infect. Dis. **11:**33–39.
14. **Grover, C. M., P. Thulliez, J. S. Remington, and J. C. Boothroyd.** 1990. Rapid prenatal diagnosis of congenital Toxoplasma infection using polymerase chain reaction in amniotic fluid. J. Clin. Microbiol. **28:**2297–2301.
15. **Guy, E. C., and H. M. Joynson.** 1995. Potential of the polymerase chain reaction in the diagnosis of active Toxoplasma infection by detection of parasite in blood. J. Infect. Dis. **172:**319–322.
16. **Hitt, J. A., and G. A. Filice.** 1992. Detection of *Toxoplasma gondii* parasitemia by gene amplification, cell culture, and mouse infection. J. Clin. Microbiol. **30:**3181–3184.
17. **Hohlfeld, P., F. Daffos, J. M. Costa, P. Thulliez, F. Forestier, and M. Vidaud.** 1994. Prenatal diagnosis of congenital toxoplasmosis with a polymerase chain reaction test on amniotic fluid. N. Engl. J. Med. **331:**695–699.
18. **Ho-Yen, D. O., A. W. Joss, A. H. Balfour, E. T. M. Smyth, D. Baird, and J. M. W. Chatterton.** 1992. Use of polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. J. Clin. Pathol. **45:**910–913.
19. **Khalifa, K. E., A. Roth, B. Roth, K. N. Arasteh, and K. Janitschke.** 1994. Value of PCR for evaluating occurrence of parasitemia in immunocompromised patients with cerebral and extracerebral toxoplasmosis. J. Clin. Microbiol. **32:**2813–2819.
20. **Lavrard, I., C. Chouaid, P. Roux, J. L. Poirot, M. Marteau, B. Lamarchand, M. C. Meyohas, and J. L. Olivier.** 1995. Pulmonary toxoplasmosis in HIV-infected patients: usefulness of polymerase chain reaction and cell culture. Eur. Respir. J. **8:**697–700.
21. **Lebech, M., A. Lebech, S. Nelsing, J. Vusst, L. Mathiesen, and E. Petersen.** 1992. Detection of *Toxoplasma gondii* DNA by polymerase chain reaction in cerebrospinal fluid from AIDS patients with cerebral toxoplasmosis. J. Infect. Dis. **165:**982–983.
22. **Liesenfeld, O., A. Roth, T. Weinke, H. D. Foss, and H. Hahn.** 1994. A case of disseminated toxoplasmosis—value of PCR for the diagnosis. J. Infect. **29:**133–138.
23. **Luft, B. J., and J. S. Remington.** 1988. Toxoplasmic encephalitis. J. Infect. Dis. **157:**1–6.
24. **Luft, B. J., and J. S. Remington.** 1992. Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis. **15:**211–222.
25. **Maniatis, T., E. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Parmley, S. F., F. D. Goebel, and J. S. Remington.** 1992. Detection of *Toxoplasma gondii* in cerebrospinal fluid from AIDS patients by polymerase chain reaction. J. Clin. Microbiol. **30:**3000–3002.
27. **Porter, S. B., and M. A. Sande.** 1992. Toxoplasmosis of the central nervous system in the acquired immunodeficiency syndrome. N. Engl. J. Med. **327:**1643–1648.
28. **Potasman, I., L. Resnick, B. J. Luft, and J. S. Remington.** 1988. Intrathecal production of antibodies against *Toxoplasma gondii* in patients with toxoplasmic encephalitis and the acquired immunodeficiency syndrome (AIDS). Ann. Intern. Med. **108:**49–51.
29. **Savva, D., J. C. Morris, J. D. Johnson, and R. E. Holliman.** 1990. Polymerase chain reaction for the detection of *Toxoplasma gondii*. J. Med. Microbiol. **32:**25–31.
30. **Smits, H. L., and R. A. Hartskeerl.** 1995. PCR amplification reactions in parasitology. J. Microbiol. Methods **23:**41–54.