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Comparison of the AF146527 and B1 Repeated Elements, Two Real-Time PCR Targets Used for Detection of *Toxoplasma gondii*▽

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Previous studies have reported the increased sensitivity of PCR targeting AF146527 over that of PCR targeting the B1 gene for diagnosis of toxoplasmosis. The present study suggests that the AF146527 element was absent in 4.8% of human *Toxoplasma gondii*-positive samples tested. The data argue that the *B1* gene may be the preferred diagnostic target.

Most infections caused by the protozoan intracellular parasite *Toxoplasma gondii* are relatively harmless and do not require medical intervention, but in some cases, infection can cause severe and even fatal disease. The outcome of an infection with *T. gondii* can depend on several factors, although only the host immune status has been proven to play an important role (8), especially when the number of CD4-positive cells has dropped below 200/μl (9). This makes toxoplasmosis one of the most common complications in HIV-infected patients, especially in sub-Saharan Africa where drugs against HIV are scarce. Early, accurate, and effective diagnosis is therefore crucial. The diagnostic method of choice is often based on detection of parasitic genomic DNA from either amniotic fluid or blood. Assays based on detection of antibodies toward the parasites are not valid for HIV-infected patients, since the titer of antibodies may be undetectable (6). Several PCR and real-time PCR assays for the detection of *T. gondii* have been developed (10). However, a range of factors may influence the diagnostic performance, e.g., the number of repeats of the target, possible polymorphism or absence of the target sequence, and the choice of oligonucleotide sequences. Real-time PCR with SYBR green or TaqMan probes has been used previously for detection and quantification of *T. gondii* parasites in different kinds of sample materials (3). Previous studies have shown that assays with multicopy targets are more sensitive for detecting *T. gondii* than those with single-copy targets (2). Two common targets used are the 35-repeat B1 gene (1) and the AF146527 sequence, a fragment that is repeated 200 to 300 times in the genome (4). Although the sensitivity of testing with the latter target has been demonstrated before, the specificity remains a subject of further investigation using a larger number of *T. gondii* strains (2).

The specificity of using the AF146527 repeat element was investigated by real-time PCR using the B1 gene as the reference. Blood samples from HIV-positive patients from East Africa were collected, and total genomic DNA was prepared as described previously (6). Alternatively, genomic DNA was purified from different parasitic strains as described earlier (7). Primer express software (Applied Biosystems) was used to optimize the design of primers and probes targeting the B1 gene and the AF146527 repeat element. For analysis of the AF146527 element, the forward primer GCTCCTCCAGCCG TCTTG, the reverse primer TCCTCACCCTCGCCTTCAT, and the TaqMan probe 6-carboxyfluorescein–AGGAGAGAT ATCAGGACTGTA–Black Hole Quencher 1 were used. The corresponding oligonucleotide sequences for analysis of the B1 gene were GCATTGCCCGTCCAAACT, AGACTGTACGGA ATGGAGACGAA, and 6-carboxyfluorescein–CAACAACTGC TCTAGCG–Black Hole Quencher 1 (Operon Biotechnologies, Germany). Real-time PCR was performed with an ABI PRISM 7900 sequence detection system (Applied Biosystems). The reaction mixtures (25 μl) consisted of 1× TaqMan PCR master mix (Applied Biosystems), 100 nM probe, and 900 nM (each) primers, forward and reverse, together with the different samples. Each well also contained 1× internal positive control (IPC) reagent and 1× IPC synthetic DNA (both from Applied Biosystems). Sterile water was used as a negative control, and *T. gondii* purified genomic DNA was used as a positive control. The amplification conditions for both B1 and AF146527 comprised 50°C for 2 min, initial activation at 95°C for 10 min, and 45 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. The amplifications of B1 and AF146527 were performed simultaneously, and samples were analyzed in triplicate. Furthermore, the B1 gene was also amplified using a PCR protocol described earlier (1).

Comparison of two different real-time PCR targets. Of 21 analyzed *T. gondii* isolates, all yielded positive PCR signals using all three protocols (two targeting the B1 gene and one targeting AF1465270). The assays demonstrated similar detection rates, and a single parasite could be detected. When the methods were tested with blood from *T. gondii*-positive HIV-infected patients as the template, a difference was seen between the two targets used. The assay targeting the AF146527

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repeat element was not able to detect parasitic DNA in 3 of 63 patient samples; meanwhile, both methods using *B1* as a target could detect parasite DNA in all 63 samples. Attempts were made to clone and sequence the repeated regions from these samples by methods described previously but with no success (4). The data indicate that there are parasite strains in which either the whole or parts of the AF146527 fragment have been deleted or mutated or in which the number of repeats vary. The latter theory is strengthened by the quantitative PCR data (not shown), which indicate that the relative proportions of AF146527 and B1 repeats differ among the isolates. Analyses of patient samples and the IPC detected no inhibitors.

Conclusion. The findings of the present study suggest that the AF146527 repeat element, with a cryptic function, was not present in all isolates analyzed; 4.8% of the samples gave false-negative results compared to results from amplification of the B1 gene. The data confirm the importance of previous recommendations to further elucidate the specificity of using a multicopy target of unknown function before the introduction of such a protocol into a diagnostic laboratory (2).

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