

## Diagnosis of Congenital Toxoplasmosis: Comparison of Targets for Detection of *Toxoplasma gondii* by PCR

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**Three PCR targets (18S ribosomal DNA, B1, and AF146527) and mouse inoculation were compared for 83 samples in the context of congenital toxoplasmosis. These four techniques are not statistically different in terms of sensitivity and specificity. However, further analysis highlighted problems sometimes encountered with PCR diagnosis of congenital toxoplasmosis.**

Primary maternal *Toxoplasma* infection exposes the fetus to the risk of parasite transmission, leading to congenital toxoplasmosis (CT), the consequences of which may be more or less severe depending on the date of transmission (7). In this situation, it is important to check for the presence or absence of the parasite in the amniotic fluid (AF) by amniocentesis 1 month after *Toxoplasma* contamination combined with mouse inoculation (MI) to compensate for the lack of absolute sensitivity of PCR (14). When it comes to selecting a target on the *Toxoplasma* DNA for PCR, the huge choice of primers reflects a lack of consensus with respect to the ideal target. However, the variations in protocols for the same target and primers (MgCl<sub>2</sub> concentration, hybridization temperature, and number of PCR cycles) are even more considerable when we look at the PCR conditions (1). In order to compare the values of different targets, we performed a comparison of the three most repeated, 18S ribosomal DNA (rDNA), B1, and AF146527, in the context of CT antenatal diagnosis and neonatal diagnosis.

**Patients.** Eighty-three samples taken from 44 newborns with mothers who had contracted toxoplasmosis during pregnancy were analyzed. Serological monitoring for toxoplasmosis in these children led to a diagnosis of CT being eliminated in 25 of them in accordance with the classification system (10). Twenty-three AF samples (including 8 taken at delivery) and 20 cord blood (CB) samples from these children were tested for *Toxoplasma* by PCR and MI. For the remaining 19 infants, serological monitoring confirmed CT (increased immunoglobulin G [IgG] content in the first 12 months of life or persistence of IgG content beyond the first 12 months of life [10] or presence of IgM after elimination of maternal contamination [3] by visualization of a profile that was different from that of the mother by Western blotting [13]). For these children, 23 AF samples (including 7 taken at delivery), 16 CB samples, and 1 peripheral venous blood sample at birth were tested.

**Methods.** DNA extraction was performed with guanidium isothiocyanate (4) or with the QIAamp DNA Mini Kit (Qiagen S.A., Courtaboeuf, France). *Taq* DNA polymerase (Gibco,

Cergy Pontoise, France) or HotStar *Taq* DNA polymerase (Qiagen S.A.) was used. The 18S rDNA (5) target was amplified with the primers described by Dupon et al. (8). The B1 target (2) was amplified with the primers described by Pelloux et al. (12). The internal control, plasmid pSYC44, verified the absence or presence of PCR inhibitors, and testing was performed separately from testing for *Toxoplasma gondii*. The AF146527 target (9) was amplified with the TOX4 and TOX5 primers (9), each without the dinucleotide CG, which is not contained in the AF146527 sequence, in the 5' position. The internal control, plasmid Tg410, verified the absence or presence of PCR inhibitors, and testing was performed separately from testing for *T. gondii*.

MI was performed as described by Pelloux and Ambroise-Thomas (11).

Results are given in Table 1 for the 19 children with CT. If a PCR result was not available because of the presence of PCR inhibitors, we considered its result negative. The use of CB seems not to be useful for the neonatal diagnosis of CT, since all tests were negative in the 19 infants with CT. For the 25 children without CT, all except one were negative for the three PCR assays and MI. The discrepant sample was an isolated positive result with B1 PCR on both AF and CB at delivery. In Table 2 are given the sensibilities and specificities referring to antenatal diagnosis and neonatal diagnosis performed either on AF or CB. Statistical tests (Fisher's exact test) performed on these performance values showed no significant difference among the three PCR assays and MI. For some cases, additional assays were performed and highlighted some problems with the PCR assays.

Case 4 illustrates post-PCR contamination. Indeed, two samples (antenatal AF 2 and neonatal AF) were positive only for 18S rDNA PCR. We then performed a *Coccidia* PCR on a different part of the 18S rDNA gene in order to determine if the DNA detected was from *Toxoplasma* or from another contaminating coccidian parasite, as the primers for 18S rDNA PCR are not specific for *Toxoplasma* (8). This *Coccidia* PCR was negative. A *Toxoplasma* real-time PCR for a new nonoverlapping target on the B1 gene (6) was negative, and JW60 enzyme-linked immunosorbent assay (ELISA) after B1 PCR hybridization with a JW60-specific probe (12; J. Weiss [Roche Molecular Systems, Alameda, Calif.], personal communica-

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TABLE 1. Comparative results of three PCR targets and MI for 19 children with congenital toxoplasmosis

Patient no.	Sample <sup>a</sup>	PCR target			MI
		18S rDNA	B1	AF146527	
1	ANAF	+	+	+	+
	NNAF	-	-	-	-
	CB	-	-	-	-
2	ANAF	+	- <sup>b</sup>	+	+
	NNAF	-	-	-	-
	CB	-	-	-	-
3	ANAF	+	+	+	+
	NNAF	+	- <sup>c</sup>	+	+
	CB	-	-	-	-
4	ANAF 1	-	-	-	-
	ANAF 2	+	-	-	-
	NNAF	+	-	-	-
	CB	-	-	-	-
5	CB	-	-	-	-
6	ANAF	-	-	-	-
	NNAF	+	- <sup>b</sup>	+	+
	CB	-	-	-	-
7	ANAF	-	-	-	-
	CB	-	-	-	-
	ANAF	-	-	-	-
8	CB	-	-	-	-
	ANAF	-	-	-	-
9	ANAF	-	-	-	-
	CB	-	-	-	-
10	ANAF	+	+	+	-
	CB	-	-	-	-
11	CB	-	-	-	-
12	NNAF	-	-	-	-
13	CB	-	- <sup>c</sup>	-	-
14	ANAF	-	- <sup>c</sup>	- <sup>c</sup>	+
	ANAF	+	+	+	+
15	CB	-	-	-	-
	ANAF	-	-	-	-
16	ANAF	+	- <sup>b</sup>	+	+
	ANAF	-	-	-	-
17	CB	-	-	-	-
	ANAF	-	-	-	-
18	ANAF	+	+	+	+
	NNAF	-	-	-	-
	CB	-	-	-	-
19	CB	-	-	-	-

<sup>a</sup> ANAF, antenatal AF; NNAF, neonatal AF; PB, peripheral blood taken at birth.

<sup>b</sup> No PCR inhibitor.

<sup>c</sup> PCR inhibitor detected.

tion) was also negative. These data indicate that the two samples did not contain *Toxoplasma* DNA and that the 18S rDNA was probably positive by post-PCR contamination.

Cases 2 (antenatal AF), 6 (neonatal AF), and 16 illustrate underdetection by B1 PCR. Only B1 PCR was negative, and

TABLE 2. PCR assay and MI performance

Assay	% Sensitivity (no./total)	% Specificity (no./total)
18S rDNA PCR	47 (9/19)	100 (23/23)
B1 PCR	26 (5/19) <sup>a</sup>	95 (21/23) <sup>c</sup>
AF146527 PCR	42 (8/19) <sup>b</sup>	100 (23/23)
MI	42 (8/19) <sup>b</sup>	100 (23/23)

<sup>a</sup> P = 0.31 (B1 PCR versus 18S rDNA PCR; Fisher's exact test).

<sup>b</sup> P = 1.00 (AF146527 PCR or MI versus 18S rDNA PCR; Fisher's exact test).

<sup>c</sup> P = 0.49 (B1 PCR versus other PCRs or MI; Fisher's exact test).

PCR inhibitors were not detected. JW60 ELISA after B1 PCR hybridization with a JW60-specific probe was positive in each case. This indicates that detection of *Toxoplasma* by B1 PCR without JW60 after PCR hybridization may lead to underdetection of the parasite.

Case 3 (neonatal AF) illustrated that a PCR inhibitor may specifically affect one target, since specific PCR inhibitor for B1 PCR was detected. Internal controls are mandatory to assess the absence or presence of inhibitors, and they need to be tested in a separate PCR (D. Filisetti, O. Villard, B. Fernique, R. Himy, C. Ruggeri, and E. Candolfi. Abstr. Congrès de la Société Française de Parasitologie, abstr. CO20, 1999). Moreover, each target has to be tested with a specific control rather than a nonspecific control (human DNA or added mouse DNA), as the inhibitor can specifically affect only one of the targets, as in case 3.

In case 13, B1 PCR and AF146527 PCR were inhibited, but PCR inhibitors could not be assessed for 18S rDNA PCR because there is no inhibitor control procedure. Positive MI in case 13 indicated that *Toxoplasma* was present.

JW60 ELISA after B1 PCR hybridization was negative on the positive B1 PCR AF at delivery and on CB in a child without CT, indicating that the positive B1 PCR was not specific for *Toxoplasma*.

Detectability of *T. gondii* using the three PCR targets with or without uracyl DNA glycosylase (UNG) (Roche Diagnostics, Meylan, France) was tested on extracted DNA (QIAamp DNA Mini Kit) after serial dilutions of the parasite. The addition of UNG did not allow amplification of DNA from one *Toxoplasma* parasite for any of the three PCR targets, whereas the lowest detectability threshold of one *Toxoplasma* parasite was reached without UNG for the three PCR targets. The use of UNG is strongly recommended, but it increases the detectability threshold from one to five parasites.

In conclusion, AF146527 PCR may be a promising new target alternative to detect *Toxoplasma* DNA in the context of CT, since there is an inhibition control procedure and performance is as good as those of other targets from a statistical point of view. Moreover, MI is an important method which should not be discontinued. In the future, it will be necessary to develop a real-time PCR based on the AF146527 target with simultaneous detection of PCR inhibitors.

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