Detection of B1 gene and REP 529 in *Toxoplasma gondii*

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Background: Toxoplasmosis is caused by the parasite *Toxoplasma gondii*. Genetic studies of *Toxoplasma gondii* isolates from Europe and the United States grouped these isolates into 3 major multi-locus genotypes, types I, II and III. In Europe, type II (haplogroup 2) and type III are predominant, but in Asia, type III (haplogroup 3) is predominant (Robert-Gangneux *et al.*, 2012).

Diagnosis of *Toxoplasma gondii* infection is normally based on serology methods, but these methods pose some difficulties for diagnosis in neonates born to infected-mothers or in immunocompromised (Table 1). A positive IgG result in a neonate may reflect maternal placental transfer, while the IgM may be detected in a neonate due to placental leakage of maternal IgM. In contrast, negative anti-*Toxoplasma* antibody does not exclude Toxoplasmosis of an immunocompromised individual.

Table 1. Diagnostic strategies for toxoplasmosis according to patient and disease setting (obtained from Robert-Gangneux *et*

Patient	Disease setting	Diagnostic approach	Technique(s)	Sample(s)
Immunocompetent patient, transplant recipient, or pregnant woman	Primary infection or determination of immune status	Serology	Routine, IgG/IgM detection[a]; complementary,[b] IgG avidity,[c] IgA detection, dye test,[d] Western blotting,[d] and ISAGA[e]	Serum
Fetus	Maternal primary infection	Prenatal diagnosis[f] based on parasite detection	PCR, mouse assay[b]	Amniotic fluid
Newborn	Maternal primary infection	Parasite detection Serology	PCR, mouse assay[b] IgG/IgM[c]/IgA detection[b] Comparative Western blotting[b]	Placenta, cord blood Cord blood serum and/or newborn serum Neonate and mother sera in parallel
Immunocompromised patient	Cerebral or disseminated toxoplasmosis	Parasite detection[b]	PCR PCR, cell culture, mouse assay, and histology	Blood CSF, BAL, tissue specimens
Immunocompetent or immunocompromised patient	Retinochoroiditis	Serology	Comparative Western blotting,[b] Goldman-Witmer coefficient[b]	Aqueous humor and serum in parallel
		Parasite detection	PCR	Aqueous humor

[a] Routine diagnosis relies mostly on ELISAs.
[b] Should be reserved for reference laboratories.
[c] For dating infection if IgM is detected, particularly in pregnant women or organ donors.
[d] When a confirmation of low IgG titers is needed.
[e] The immunosorbent agglutination assay is a reference technique to confirm IgM specificity and to detect IgM in congenitally infected neonates.
[f] Ministerial agreement is required in some countries.

Thus, direct detection of *Toxoplasma gondii* DNA using PCR, targeting the 35-fold repeated B1 gene has been commonly used for molecular diagnosis (Burg JL *et al.*, 1989), but another sequence, the 529-bp repeat element (REP 529) was described more recently as being 200 to 300-fold repeated (Homan WL *et al.*, 2000). Hence, two real-time PCR, one targeting B1 gene and the other targeting REP 529, were used to detect the genome of *Toxoplasma gondii.*

Materials/methods: 39 archived samples were selected. 25 were proficiency samples from QCMD (17 type II positives, 8 negatives) and 14 were clinical samples (3 unknown positives, 11 negatives). These 39 samples were extracted by EZ1 Virus mini kit v2.0 (QIAGEN) and amplified on CFX96 (Bio-Rad) using QuantiTect Multiplex No Rox Kit (QIAGEN) (Table 2). A quantitated, purified complete genome of *Toxoplasma gondii* DNA control, (Amplirun *Toxoplasma gondii* DNA Control, Vircell) was diluted to test the limit of detection.

Table 2. Primer and probe sequences used for *Toxoplasma gondii* RT-PCR

Primer	Sequence (5'-3')	Size	
TG B1 F	GAA AGC CAT GAG GCA CTC CA	98 bp	Belaz S *et al.*, 2015
TG B1 R	TTC ACC CGG ACC GTT TAG C		
TG B1 Probe	HEX-CGG GCG AGT AGC ACC TGA GGA GAT ACA-BHQ1		
TG REP-529 270F	AgAgACACCggAATgCgATCT	112 bp	Robert-Gangneux *et al.*, 2015
TG REP-529 381R	TTCgTCCAAgCCTCCgACT		
TG REP-529 310 Probe	6-FAM-TCgTggTgATggCggAgAgAATTgA-BHQ1		

Results: 76.4% (13/17) of the archived proficiency positive samples were detected by both *Toxoplasma gondii* B1 gene and REP 529 after re-extraction (Table 3). The original extracts of QCMD 2013 13-01, 13Reg-01 and 13Reg-02 were detected by both *Toxoplasma gondii* B1 gene and REP 529, however they were detected by either *Toxoplasma gondii* B1 gene or 529 bp repeat element after re-extraction likely due to freeze-thaw degradation. The original extract of QCMD 2014 14-01 was not available for verification. This could also be due to freeze-thaw degradation.

100% (3/3) of the clinical positives were detected by both *Toxoplasma gondii* B1 gene and REP 529 after re-extraction. 100% (8/8) of the archived proficiency negative samples and 100% (11/11) of the clinical negatives were also not detected by both *Toxoplasma gondii* B1 gene and 529 bp repeat element after re-extraction. A quantitated, purified complete genome of *Toxoplasma gondii* DNA control, (Amplirun *Toxoplasma gondii* DNA Control, Vircell) was diluted to test the limit of detection. This assay can detect at 95% confidence level, B1 gene at 0.97 copies/µL and REP 529 at 0.69 copies/µL, based on probit analysis (Table 4).

Table 3. Real time-PCR results of *Toxoplasma gondii*, targeting B1 gene and REP 529 on EQA samples

Toxoplasma gondii (EQA)		Re-extracted samples		Repeat with orginal extract	
		B1	REP 529	B1	REP 529
QCMD 2013 13-01	Positive	Detected	Not detected	Detected	Detected
QCMD 2013 13-04	Positive	Detected	Detected		
QCMD 2013 13-09	Positive	Detected	Detected		
QCMD 2013 13Reg-01	Positive	Not detected	Detected	Detected	Detected
QCMD 2013 13Reg-02	Positive	Detected	Detected		
QCMD 2013 13Reg-03	Positive	Not detected	Detected	Detected	Detected
QCMD 2014 14-01	Positive	Not detected	Detected	Not available	
QCMD 2014 14-02	Positive	Detected	Detected		
QCMD 2014 14-03	Positive	Detected	Detected		
QCMD 2014 14-05	Positive	Detected	Detected		
QCMD 2014 14-06	Positive	Detected	Detected		
QCMD 2014 14-07	Positive	Detected	Detected		
QCMD 2014 14-08	Positive	Detected	Detected		
QCMD 2014 14Reg-02	Positive	Detected	Detected		
QCMD 2014 14Reg-03	Positive	Detected	Detected		
QCMD 2014 14Reg-04	Positive	Detected	Detected		
QCMD 2013 13-06	Positive	Detected	Detected		
QCMD 2013 13-03	Negative	Not detected	Not detected		
QCMD 2013 13-07	Negative	Not detected	Not detected		
QCMD 2013 13-08	Negative	Not detected	Not detected		
QCMD 2013 13Reg-04	Negative	Not detected	Not detected		
QCMD 2014 14-04	Negative	Not detected	Not detected		
QCMD 2014 14-09	Negative	Not detected	Not detected		
QCMD 2014 14-10	Negative	Not detected	Not detected		
QCMD 2014 14Reg-01	Negative	Not detected	Not detected		

Table 4. Confidence limits of detecting *Toxoplasma gondii*

Probability	95% Confidence Limits (copies/ µL)	
	B1 gene	REP 529
PROBIT 0.900	0.77	0.33
0.950	0.97	0.69
0.990	1.35	1.38

Conclusions: B1 gene and REP 529 can detect *Toxoplasma gondii* in clinical and proficiency samples, but REP 529 is slightly more sensitive than B1 gene.

References:

1. Burg JL et al. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii, by polymerase chain reaction.* J Clin Microbiol. 1989 27:1787-1792
2. Homan WL *et al.* Identification of a 200 to 300-fold repetitive 529bp DNA fragment in *Toxoplasma gondii,* and its use for diagnostic and quantitative PCR. Int J Parasitol. 2000 30:69-75
3. Belaz S, Gangneux JP, Dupretz P, Guiguen C, Robert-Gangneux F. A 10-year retrospective comparison of two target sequences, REP-529 and B1, for *Toxoplasma gondii* detection by quantitative PCR. J Clin Microbiol. 2015 Apr;53(4):1294-300
4. Robert-Gangneux F, Dupretz P, Yvenou C, Quinio D, Poulain P, Guiguen C, Gangneux JP. Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. Pediatr Infect Dis J. 2010 Jan;29(1):33-8.