


RESEARCH ARTICLE

Open Access



Comparison of an *in house* and a commercial real-time polymerase chain reaction targeting *Toxoplasma gondii* RE gene using various samples collected from patients in Turkey

Mert Döşkaya¹, Hüsnü Pullukçu², Muhammet Karakavuk^{1,3}, Esra Atalay Şahar^{1,4}, Mehmet Sezai Taşbakan⁵, Meltem Işıkgöz Taşbakan², Mümtaz Yılmaz⁶, Hüseyin Can⁷, Aysu Değirmenci Döşkaya^{1*}  and Adnan Yüksel Gürüz¹

Abstract

Background: *Toxoplasma gondii* is an opportunistic protozoan parasite that can infect all warm-blooded animals including humans and cause serious clinical manifestations. Toxoplasmosis can be diagnosed using histological, serological, and molecular methods. In this study, we aimed to detect *T. gondii* RE gene in various human samples by *in house* and commercial real time polymerase chain reactions.

Methods: A total of 38 suspected cases of toxoplasmosis [peripheral blood (n:12), amnion fluid (n:11), tissue (n:9), cerebrospinal fluid (n:5), and intraocular fluid (n:1)] were included to the study. An *in house* and a commercial RT-PCR were applied to investigate the *T. gondii* RE gene in these samples.

Results: The compatibility rate of the two tests was 94.7% (37/38). When the commercial RT-PCR kit was taken as reference, the sensitivity and specificity of *in house* RT-PCR test was 87.5 and 100%. When the *in house* RT-PCR test was taken as reference, the commercial RT-PCR kit has 100% sensitivity and 96.8% specificity. Incompatibility was detected in only in a buffy coat sample with high protein content.

Conclusions: Both the commercial and *in house* RT-PCR tests can be used to investigate *T. gondii* RE gene in various clinical specimens with their high sensitivity and specificity. In house RT-PCR assay can be favorable due to cost savings compared to using the commercial test.

Keywords: *Toxoplasma gondii*, RE gene, Diagnosis, Real time PCR, *In house*

Background

Toxoplasma gondii is an opportunistic protozoan parasite that can infect all warm-blooded animals including birds and can cause serious clinical manifestations. *T. gondii* causes congenital toxoplasmosis leading to fetal anomalies in newborns, retinochoroiditis causing blindness, deadly toxoplasmic encephalitis in immunocompromised patients or transplant recipients [1, 2].

Toxoplasmosis can be diagnosed using histological, serological, and molecular methods [1, 3–6]. In recent years, serological and molecular diagnostic methods are frequently used. Serological screening for toxoplasmosis is performed in pregnant women in most of the countries during the first trimester. When IgM positivity and low IgG avidity are detected in the pregnant woman, there is a risk of congenital toxoplasmosis in the fetus [1]. In order to rule out this suspicion, amniocentesis is recommended during the 16–18th weeks of gestation and amniotic fluid is investigated with Polymerase chain reaction (PCR) to detect *T. gondii* DNA [1, 7]. In addition, molecular techniques become important due to

* Correspondence: aysu.degirmenci.doskaya@ege.edu.tr

¹Department of Parasitology, Ege University Faculty of Medicine, 35100 Izmir, Bornova, Turkey

Full list of author information is available at the end of the article



decreased levels of antibodies in immune suppressed patients [8–10].

The majority of *T. gondii* strains from most parts of the world belong to three different clonal clade, called type I, type II and type III. There are also atypical and recombinant strains present [11–15]. The *T. gondii* B1 gene (GenBank no: AF179871) containing 35 copies, the RE gene (GenBank no: AF146527) containing 200–300 copies and the SAG1 (GenBank: M23658) gene consisting of single copy were used in molecular diagnostic tests [16–19]. In a recent study, the copy numbers for the B1 gene were found to be 5, 12 and 7 times lower than the previous estimations of 35 copies for type I, type II and Type III strains, respectively. For *T. gondii* RE gene were found to be 8, 4 and 4 times lower than the previous estimations of 200–300 copies for type I, type II and Type III strains, respectively [17]. PCR studies performed in various clinical samples showed that *T. gondii* RE gene was more sensitive and specific than B1 gene [20–29]. Moreover *Toxoplasma* Reference centers in Europe are using PCR reactions targeting *T. gondii* RE gene more frequently compared to *T. gondii* PCR targeting B1 gene [30, 31].

In this study, two hybridization probe based methods which are an *in house* Real Time PCR and a commercial Real Time PCR targeting *T. gondii* RE gene were compared in terms of sensitivity and specificity using different clinical samples.

Methods

Clinical samples

In this study, 38 specimens [12 peripheral blood sample, 11 amniotic fluid, 9 tissue, 5 cerebrospinal fluid (CSF) and 1 intraocular fluid] obtained from 38 clinically toxoplasmosis suspected seropositive patients admitted to Ege University Faculty of Medicine, were investigated for the presence of *T. gondii* using an *in house* Real Time PCR and a commercial Real Time PCR targeting RE gene.

Specimen processing and DNA extraction

DNA extraction from amniotic fluid, peripheral blood, tissue, CSF, or intraocular fluid sample was performed using the Qiagen Mini Kit according to the manufacturer's protocol. Initially, specimens were processed. During the DNA extraction of blood sample, the buffy coat part was used [31–33]. For this purpose, 2–4 ml whole blood in EDTA was centrifuged at 3000 rpm for 15 min. After centrifugation, plasma was discharged until ~ 300 µl plasma remained. Then, 500 µl of sample was used for DNA extraction [~ 300 µl plasma (+) ~ 100 µl buffy coat (+) ~ 100 µl of erythrocyte cluster]. During the extraction of DNA from amniotic fluid, at least 6 ml sample was used [34]. For a sample more than 6 ml,

whole specimen was transferred to a sterile 15 ml tube and centrifuged at 3000 rpm for 15 min. Next, the supernatant was discarded until ~ 6 ml amniotic fluid remained. For the tissue, 50 mg sample was finely chopped and added to tube containing ATL buffer, 80 µl Proteinase K, 0,1 mm glass beads, and 2 mm zirconia beads (BioSpec Products, U.S.A.) and incubated for 15 min at 56 °C with 1400 rpm in a heat shaker (Lab4You) until the sample melted. Next, the tissue samples were incubated at 95 °C for 5 min. After incubation, samples were vortexed for 2 min on a Disruptor Genie (Scientific Industries, U.S.A.) [35, 36]. CSF and intraocular fluid samples were used directly when the volume of sample was ~ 200 µl, and in the case of excess, the sample was centrifuging at 3000 rpm and the supernatant was discarded until ~ 200 µl sample remained.

Real time PCR

In house Real Time PCR to detect 134 bp region of *T. gondii* RE gene (GenBank no: AF146527) was performed as described with some minor modifications [20]. The analysis of results was performed by 1.5 LightCycler Real Time instrument using LightCycler software, Version 3.5 according to the manufacturers protocol (Roche). Twenty microliter final volume PCR reaction included 1x LightCycler Fast Start DNA Master HybProbe mix with MgCl₂ final concentration adjusted to 5 mM (Roche), the primers 5'-AGGCGAGGGTGAGGATGA-3' (18 nt, TOX-SE forward primer; final concentration: 0.5 µM) and 5'-TCGTCTCGTCTGGATCGCAT-3' (20 nt, TOX-AS reverse primer; final concentration: 0.5 µM), the hybridization probes 5'-GCCGAAACATCTTCTCCCTCTCC-3'-FL (24 nt, TOX FLU, labeled at the 3' end with fluorescein; final concentration: 0.1 µM) and 5'-640-CTCTCGTCGCTTCCCAACCACG-3' (22 nt, TOX LCR labeled at the 5' end with LC-Red 640; final concentration: 0.5 µM) (IDT), DNA template or controls. The PCR amplification reactions were performed by the following calculated protocol: 10 min initial denaturation step at 95 °C, followed by 50 cycles of 5 s at 95 °C, 10 s at 60 °C, and 15 s at 72 °C. PCR reactions were performed in duplicate.

As positive and negative controls, *T. gondii* genomic DNA serially 10-fold diluted ranging from 10⁶ to 10¹ parasites/µl and distilled water were used, respectively. In clinical samples and positive controls, crossing point (Cp) values were used to assess the amount of *T. gondii* DNA. Melting curve analysis was performed using the following calculated protocol: 20 s denaturation step at 95 °C followed by 20 s annealing step at 40 °C and extension step gradually increasing temperature to 85 °C.

To analyze the PCR inhibition attributable to the specimen matrix, PCR reactions were prepared in duplicate for each sample. One of the reactions contained only

purified patient DNA sample and the other reaction contained 1 *T. gondii* tachyzoite spiked into the purified patient sample. The sample is accepted as inhibited when the spiked control did not show any crossing point (Cp) value.

The commercial *T. gondii* Real Time PCR kit (TIB-MolBiol) targeting 134 bp fragment of *T. gondii* RE gene was applied according to the manufacturer's protocol. The analysis of results was performed by 1.5 LightCycler Real Time instrument using LightCycler software, Version 3.5 according to the manufacturers protocol (Roche). In this test, the internal control target was investigated as multiplex. The control reaction mix contained the primer and the probe mix that determines the internal control, while the reaction also included the lambda phage as the extraction control target. Twenty microliter final volume PCR reaction included 1x LightCycler Fast Start DNA Master HybProbe mix with MgCl₂ final concentration adjusted to 4.5 mM (Roche), 2 µl PSR mix (parameter specific reagents; containing *T. gondii* RE gene specific primers and hybridization probes), DNA template or controls, 2 µl control target (lambda phage) and 0.5 µl control reaction mix (contains primers and probe for lambda phage). The PCR amplification reactions were performed by the following calculated protocol: 10 min initial denaturation step at 95 °C, followed by 50 cycles of 5 s at 95 °C, 5 s at 62 °C, and 15 s at 72 °C. Moreover, a single cycle of 55 °C with 0.5 step size was included to the amplification step. PCR reactions were performed in duplicate.

As positive and negative controls, *T. gondii* plasmid containing 134 bp fragment of RE gene serially 10-fold diluted ranging from 10⁶ to 10¹ copy plasmids/reaction and distilled water were used. In clinical samples and positive controls, crossing point (Cp) values were used to assess the amount of *T. gondii* DNA. Melting curve analysis was performed using the following calculated protocol: 20 s denaturation step at 95 °C followed by 20 s

annealing step at 40 °C and extension step gradually increasing temperature to 85 °C.

Results

According to the results, among the 38 clinical samples analyzed, *T. gondii* RE gene was not detected in 30 of them and *T. gondii* RE gene was detected in 6 samples by both *in house* Real Time PCR and commercial Real Time PCR (Table 1). Regarding the remaining two samples; one sample was partly compatible in both assays in which *T. gondii* RE gene was detected during the first run and not detected in the subsequent using *in house* Real Time PCR, whereas the commercial Real Time PCR was positive in both runs for this sample. In the remaining sample, the results were completely incompatible between both assays (Table 1). Both of these samples were buffy coat samples. As a result, the compatibility rate of both tests was 97.4% (37/38) after two tests.

The sensitivity of the *in house* Real Time PCR was 87.5% (7/8) and the specificity was 100% (30/30) when we take commercial Real Time PCR as the golden standard. Conversely, if we take *in house* Real Time PCR as the golden standard, the sensitivity and specificity of the commercial Real Time PCR was 100% (7/7) and 96.8% (30/31), respectively. Due to low sample size, individual sensitivity and specificity values were not calculated.

As the results of the melting curve analysis of positive control and positive clinical samples were examined, it was found that T_m of all samples peaked around 67.5 ± 1 °C using *in house* and commercial Real Time PCRs. When the internal control data were examined, any inhibition was not detected in both tests. As the positive controls were examined, it was found that the analytical sensitivity of the commercial Real Time PCR was 10 copy plasmid/reaction and the analytical sensitivity of the *in house* Real Time PCR was 1 parasite/reaction. No

Table 1 Comparison of in house and commercial Real Time PCR kits targeting *T. gondii* RE gene in 38 clinical samples

Sample type	<i>in house</i> Real Time PCR	Commercial Real Time PCR	Compatibility	Number of sample with the same PCR results
Peripheral blood (Buffy coat)	-/-	-/-	Compatible	8
	+/+	+/+	Compatible	2
	-/-	+/+	Partly compatible	1
	+/-	+/+	Completely incompatible	1
Amniotic fluid	-/-	-/-	Compatible	10
	+/+	+/+	Compatible	1
Tissue	-/-	-/-	Compatible	6
	+/+	+/+	Compatible	3
Cerebrospinal fluid (CSF)	-/-	-/-	Compatible	5
Intraocular fluid	-/-	-/-	Compatible	1
			TOTAL	38

signal was detected in the distilled water negative control samples.

Discussion

Molecular diagnostic tests have high sensitivity and specificity in diagnosing congenital toxoplasmosis, toxoplasmic retinochoroiditis, as well as organ transplantation and AIDS patients [37]. *T. gondii* B1 and RE genes are the most frequently targeted markers in the molecular diagnosis of toxoplasmosis due to their high copy numbers [16, 17]. Some studies have shown that the *T. gondii* RE gene is more sensitive and specific than the B1 gene [20–30]. In East Africa, blood samples of 63 patients were investigated with Real Time PCR methods targeting the B1 and RE genes of *T. gondii*. Interestingly, B1 gene was detected in all cases known to be positive for *T. gondii*, whereas in 3 samples (4.8%), RE gene could not be detected. The authors stated that the G/C mutation at position 275 of the RE gene inhibited the binding of the primer to the RE gene and affected real time PCR results. In addition, the genotypes detected in Africa are different from Europe. The higher ratio of RE/B1 copy number detected in type II strains which is more common in Europe has been shown to be a cause of the high specificity and success of real time PCR targeting the RE gene in Europe [29].

In Turkey, two cases of congenital toxoplasmosis detected in 1972 and 2007 have been isolated from two infants and has been shown to be similar to the isolates of African genotype 1 [38]. In addition, two studies conducted in big groups of cats and wild birds of Turkey, Type II and III strains are found to be more frequent like in Europe [14, 15]. In our country, more frequent detection of type II strains in animals suggests that PCR targeting the *T. gondii* RE gene may be more sensitive. Thus, in this study, two very similar hybridization probe based methods which are an *in house* Real Time PCR described by Cassaigne et al., 2006 and a commercial Real Time PCR kit targeting the same region of *T. gondii* RE gene were first time compared in terms of sensitivity and specificity using various clinical samples.

A total of 38 suspected cases of toxoplasmosis were included to the study. The reason for low sample size can be attributed to presence of *T. gondii* in blood approximately for only 2 weeks during acute infection of immunocompetent patients [1, 2]. Besides, in a patient infected with *T. gondii*, seroconversion (i.e. IgG positivity) takes place around 15 days and as the clinicians order to perform *T. gondii* PCR, tachyzoites may become latent in tissues (also called chronic toxoplasmosis) and not found in blood circulation [1, 2]. In addition, an important clinical presentation called congenital toxoplasmosis is a rare disease according to the Oprhanet report series [39]. Overall, *T. gondii* DNA positivity may not be

frequently detected in blood or amniotic fluid samples of toxoplasmosis cases. Thus, although we aimed to evaluate more *T. gondii* DNA positive samples in this study, we couldn't achieve it and the low *T. gondii* DNA positive sample size can affect the interpretation of the sensitivity ratio which is the limitation of this study.

The results showed that the compatibility rate of the *in house* Real Time PCR and commercial Real Time PCR targeting the *T. gondii* RE gene was 97.4% using 38 different clinical samples. Among the 38 samples analyzed in this study, 36 of them (six positive and 30 negative) were found to be fully compatible, while one buffy coat sample was partly compatible and the other was completely incompatible (Table 1). In these two samples, the UV260/280 ratio was > 1.8 and no inhibition was observed. In one sample, the *in house* Real Time PCR was one time negative and the other time positive which can be attributed to a low *T. gondii* DNA in the sample (Table 1). In this sample, the Cp value of the *in house* Real Time PCR was 36, supporting the difference among the results. Similarly, with the commercial Real Time PCR, the Cp value of 34.6 supports the fact that the concentration of *T. gondii* RE gene was low. Consequently, the commercial Real Time PCR kit can be considered to be more sensitive in detecting the *T. gondii* RE gene. In this situation, the problem of hypersensitivity of the PCR may occur leading to false positivity due to forced cross-linking of primers and probes to primer dimers at the last cycles of the PCR. In this study, the Cp value of the buffy coat sample (which was found two times negative with *in house* Real Time PCR and two times positive with commercial Real Time PCR kit) as detected by commercial Real Time PCR kit were 34.3 and 34 and can be evaluated as hypersensitivity. Conversely, the inaccurate negative result as detected by *in house* Real Time PCR may be due to the loss of sensitivity because of high protein content in buffy coat. In a study, the buffy coat samples of 44 patients with clinically proven acute toxoplasmosis was investigated in two different centers by hybridization probe based Real Time PCR assay targeting the *T. gondii* RE gene. Both centers found 25% (9/44) positivity. Only four of these nine patients were found to be positive in both centers, while four samples were positive in one center and one sample positive in another center [33]. As a result, buffy coat samples can be considered as challenging samples for the detection of *T. gondii* DNA.

Conclusions

Both tests were compatible with amniotic fluid, tissue, CSF or intraocular fluid samples, and showed complete incompatibility only in a buffy coat sample with a dense protein content. In particular, it may be useful to analyze high protein content samples such as buffy coat twice to

detect low amount of *T. gondii* DNA. As a result, the high compatibility, analytical sensitivity, sensitivity, and specificity of both tests suggest that the Real Time PCR methods targeting *T. gondii* RE gene can be used to investigate *T. gondii* DNA in various clinical samples. *In house* real time PCR can be preferred by developing laboratories due to cost savings.

Abbreviations

CSF: Cerebrospinal fluid; DNA: Deoxyribonucleic acid; IgG: Immune globulin G; IgM: Immune globulin M; min: minutes; rpm: rounds per minute; PCR: Polymerase chain reaction; RE: Repeat element; RT-PCR: Real-time polymerase chain reaction; *Toxoplasma gondii*: *T. gondii*

Acknowledgements

The authors would like to acknowledge *Toxoplasma* Biological Resource Center (BRC)/Centre National de Référence (CNR) *Toxoplasma* for their technical help in development of the *in house* Real Time PCR method.

Authors' contributions

MD, EAŞ, MK, HC, ADD, AYG conceived and designed the study. HP, MST, MIT, MY worked in acquisition of the clinical samples. MD, HP, MK, EAŞ, MST, MY, ADD, HC helped in interpretation of data. MD, AYG performed the statistical analyses. MD, ADD, MK, EAŞ, MIT, HC interpreted the results and worked in drafting the manuscript. MD and ADD participated in revising the manuscript. All authors read and approved the final manuscript.

Funding

This study was partly supported by the grant given by the Scientific Research Projects Branch Directorate of Ege University, Turkey (Grant No: 2013-TIP-050) to Y.G. The funding body does not have any role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All samples were collected with patient written consent as approved by the Research Ethics Committee of the Ege University Faculty of Medicine (Approval number: 13–3/3; 18–5.1/29).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Parasitology, Ege University Faculty of Medicine, 35100 İzmir, Bornova, Turkey. ²Department of Infectious Diseases, Ege University Faculty of Medicine, İzmir, Bornova, Turkey. ³Ege University, Ödemiş Vocational High School, İzmir, Ödemiş, Turkey. ⁴Department of Biotechnology, Ege University Faculty of Engineering, İzmir, Bornova, Turkey. ⁵Department of Chest Diseases, Ege University Faculty of Medicine, İzmir, Bornova, Turkey. ⁶Department of Internal Medicine, Ege University Faculty of Medicine, İzmir, Bornova, Turkey. ⁷Department of Biology, Molecular Biology Section, Ege University Faculty of Sciences, İzmir, Bornova, Turkey.

Received: 15 July 2019 Accepted: 29 November 2019

Published online: 10 December 2019

References

- Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet*. 2004;363:1965–76.
- Weiss LM, Kim K. *Toxoplasma gondii*, the model Apicomplexan: perspectives and methods. Great Britain: Elsevier Ltd; 2007.
- Smit GSA, Vu TLB, Do TD, Speybroeck N, Devleeschauwer B, Padalao E, et al. Prenatal diagnosis and prevention of toxoplasmosis in pregnant women in northern Vietnam: study protocol. *BMC Infect Dis*. 2017;17:364.
- Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev*. 2012;25:264–96.
- Dogan N, Akda İ, Essizoglu A, Güleç G. Bipolar afektif bozukluk ve şizofreni hastalarında *Toxoplasma gondii* varlığının ve ilişkisinin serolojik ve moleküler yöntemlerle araştırılması. *Flora*. 2018;23(3):142–9.
- Gashout A, Amro A, Erhuma M, Al-Dwibe H, Elmahub E, Babba H, Nattah N, Abudher A. Molecular diagnosis of toxoplasma gondii infection in Libya. *BMC Infect Dis*. 2016;16:157.
- Thangarajah P, Hajissa K, Wong WK, Abdullah MA, Ismail N, Mohamed Z. Usefulness of paired samples for the Serodiagnosis of toxoplasmosis infection in a tertiary teaching Hospital in Malaysia. *BMC Infect Dis*. 2019;19:202.
- Derouin F, Pelloux H. ESCMID study group on clinical parasitology. Prevention of toxoplasmosis in transplant patients. *Clin Microbiol Infect*. 2008;14:1089–101.
- Martino R, Maertens J, Bretagne S, Rovira M, Deconinck E, Ullmann AJ, et al. Toxoplasmosis after hematopoietic stem cell transplantation. *Clin Infect Dis*. 2000;31:1188–95.
- Fricker-Hidalgo H, Bulabois CE, Brenier-Pinchart MP, Hamidfar R, Garban F, Brion JP, et al. Diagnosis of toxoplasmosis after allogeneic stem cell transplantation: results of DNA detection and serological techniques. *Clin Infect Dis*. 2009;48:e9–15.
- Dardé ML. *Toxoplasma gondii*, “new” genotypes and virulence. *Parasite*. 2008;15:366–71.
- Ajzenberg D, Yera H, Marty P, Paris L, Dalle F, Menotti J, et al. Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. *J Infect Dis*. 2009;199:1155–67.
- Delhaes L, Ajzenberg D, Sicot B, Bourgeot P, Dardé ML, Dei-Cas E, et al. Severe congenital toxoplasmosis due to a *Toxoplasma gondii* strain with an atypical genotype: case report and review. *Prenat Diagn*. 2010;30:902–5.
- Can H, Döşkaya M, Ajzenberg D, Özdemir HG, Caner A, İz SG, et al. Genetic characterization of *Toxoplasma gondii* isolates and toxoplasmosis seroprevalence in stray cats of İzmir, Turkey. *PLoS One*. 2014;9:e104930.
- Karakavuk M, Aldemir D, Mercier A, Atalay Şahar E, Can H, Murat JB, et al. Prevalence of toxoplasmosis and genetic characterization of *Toxoplasma gondii* strains isolated in wild birds of prey and their relation with previously isolated strains from Turkey. *PLoS One*. 2018;13:e0196159.
- Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *Int J Parasitol*. 2000;30:69–75.
- Costa JM, Bretagne S. Variation of B1 gene and AF146527 repeat element copy numbers according to *Toxoplasma gondii* strains assessed using real-time quantitative PCR. *Clin Microbiol*. 2012;50:1452–4.
- Burg JL, Perelman D, Kasper LH, Ware PL, Boothroyd JC. Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii*. *J Immunol*. 1988;141:3584–91.
- Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J Clin Microbiol*. 1989;27:1787–92.
- Cassaing S, Bessières MH, Berry A, Berrebi A, Fabre R, Magnaval JF. Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. *J Clin Microbiol*. 2006;44:720–4.
- Kasper DC, Sadeghi K, Prusa AR, Reischer GH, Kratochwill K, Förster-Waldl E, et al. Quantitative real-time polymerase chain reaction for the accurate detection of *Toxoplasma gondii* in amniotic fluid. *Diagn Microbiol Infect Dis*. 2009;63:10–5.
- Menotti J, Garin YJ, Thulliez P, Sérugue MC, Stanislawiak J, Ribaud P, et al. Evaluation of a new 5'-nuclease real-time PCR assay targeting the *Toxoplasma gondii* AF146527 genomic repeat. *Clin Microbiol Infect*. 2010;16:363–8.
- Filissetti D, Gorcii M, Pernot-Marino E, Villard O, Candolfi E. Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. *J Clin Microbiol*. 2003;41:4826–8.
- Reischl U, Bretagne S, Krüger D, Ernault P, Costa JM. Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. *BMC Infect Dis*. 2003;3:7.

25. Edvinsson B, Lappalainen M, Evengård B. ESCMID study Group for Toxoplasmosis. Real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. *Clin Microbiol Infect.* 2006;12:131–6.
26. Sterkers Y, Varlet-Marie E, Cassaing S, Brenier-Pinchart MP, Brun S, Dalle F, et al. Multicentric comparative analytical performance study for molecular detection of low amounts of *Toxoplasma gondii* from simulated specimens. *J Clin Microbiol.* 2010;48:3216–22.
27. 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;53:1294–300.
28. Filisetti D, Sterkers Y, Brenier-Pinchart MP, Cassaing S, Dalle F, Delhaes L, et al. Multicentric comparative assessment of the bio-evolution *Toxoplasma gondii* detection kit with eight laboratory-developed PCR assays for molecular diagnosis of congenital toxoplasmosis. *J Clin Microbiol.* 2015;53:29–34.
29. Wahab T, Edvinsson B, Palm D, Lindh J. Comparison of the AF146527 and B1 repeated elements, two real-time PCR targets used for detection of *Toxoplasma gondii*. *J Clin Microbiol.* 2010;48:591–2.
30. Roux G, Varlet-Marie E, Bastien P, Sterkers Y. French National Reference Center for toxoplasmosis network. Evolution of *Toxoplasma*-PCR methods and practices: a French national survey and proposal for technical guidelines. *Int J Parasitol.* 2018;48:701–7.
31. Robert-Gangneux F, Brenier-Pinchart MP, Yera H, Belaz S, Varlet-Marie E, Bastien P; molecular biology study Group of the French National Reference Center for toxoplasmosis. Evaluation of *Toxoplasma* ELITE MGB real-time PCR assay for diagnosis of toxoplasmosis. *J Clin Microbiol.* 2017;55:1369–76.
32. Brenier-Pinchart MP, Capderou E, Bertini RL, Bailly S, Fricker-Hidalgo H, Varlet-Marie E, et al. Molecular diagnosis of toxoplasmosis: value of the buffy coat for the detection of circulating *Toxoplasma gondii*. *Diagn Microbiol Infect Dis.* 2015;82:289–91.
33. Ajzenberg D, Lamaury I, Demar M, Vautrin C, Cabié A, Simon S, et al. Performance testing of PCR assay in blood samples for the diagnosis of Toxoplasmic encephalitis in AIDS patients from the French Departments of America and genetic diversity of *Toxoplasma gondii*: a prospective and multicentric study. *PLoS Negl Trop Dis.* 2016;10:e0004790.
34. Morelle C, Varlet-Marie E, Brenier-Pinchart MP, Cassaing S, Pelloux H, Bastien P, et al. Comparative assessment of a commercial kit and two laboratory-developed PCR assays for molecular diagnosis of congenital toxoplasmosis. *J Clin Microbiol.* 2012;50:3977–82.
35. Döşkaya M, Caner A, Degirmenci A, Wengenack NL, Yolasigmaz A, Turgay N, et al. Degree and frequency of inhibition in a routine real-time PCR detecting *Pneumocystis jirovecii* for the diagnosis of *Pneumocystis* pneumonia in Turkey. *J Med Microbiol.* 2011;60:937–44.
36. Karakavuk M, Aykur M, Şahar EA, Karakuş M, Aldemir D, Döndüren Ö, et al. First time identification of *Acanthamoeba* genotypes in the cornea samples of wild birds; is *Acanthamoeba* keratitis making the predatory birds a target? *Exp Parasitol.* 2017;183:137–42.
37. Remington JS, Thulliez P, Montoya JG. Recent developments for diagnosis of toxoplasmosis. *J Clin Microbiol.* 2004;42:941–5.
38. Döşkaya M, Caner A, Ajzenberg D, Değirmenci A, Dardé ML, Can H, et al. Isolation of *Toxoplasma gondii* strains similar to Africa 1 genotype in Turkey. *Parasitol Int.* 2013;62:471–4.
39. List of rare diseases and synonyms - Orphanet. https://www.orpha.net/orphacom/cahiers/docs/GB/List_of_rare_diseases_in_alphabetical_order.pdf. Accessed 23 Oct 2019.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

