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ORIGINAL PAPER

Challenging TaqMan probe-based real-time PCR and loop-mediated isothermal amplification (LAMP): the two sensitive molecular techniques for the detection of toxoplasmosis, a potentially dangerous opportunistic infection in immunocompromised patients

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Abstract

Due to defects and drawbacks of most conventional diagnostic methods including serology for the diagnosis of toxoplasmosis as a dangerous opportunistic infection in immunocompromised individuals, the accurate, rapid, and sensitive detection of infection in such patients is essential. In this study, the TaqMan probe-based real-time PCR and, a relatively new nucleic acid amplification method, the loop-mediated isothermal amplification (LAMP) technique was compared based on the repetitive elements (RE) sequence to detect *Toxoplasma gondii* (*T. gondii)* DNA in blood samples of immunocompromised individuals. During this study, 119 blood samples from immunocompromised cancer patients with renal failure, undergoing dialysis were studied. After DNA extraction from blood samples using the salt extraction method, the molecular techniques of TaqMan probe-based real-time PCR and LAMP were used to investigate the contamination of the samples with *T. gondii,* based on the 529 bp (RE) sequence of *T. gondii*. The analytical sensitivity of LAMP and real-time PCR was evaluated by duplicating the five-step serial dilutions of *T. gondii* tachyzoites from 0.25 to 5×10^5 spiked tachyzoites per milliliter of the *Toxoplasma* seronegative blood sample. The extracted DNA from other parasites and human chromosomal DNA were used to determine the specificity of the molecular methods. The obtained results were analyzed using Kappa statistical test and SPSS22 software. Out of 119 studied samples, 7 (5.8%) and 5 (4.2%) samples were positive for *Toxoplasma* by TaqMan probe-based real-time PCR and LAMP, respectively. The limits of detection of TaqMan probe-based real-time PCR and RE-LAMP in negative serum samples were one and five tachyzoites (CT 38), respectively. Both real-time PCR and LAMP methods were 100% specific for *Toxoplasma* detection. Positive results were obtained only with *T. gondii* DNA, while other DNA samples were negative. The TaqMan probe-based real-time PCR based on the RE sequence showed higher sensitivity to *T. gondii* DNA detection in blood samples of cancer patients and serial dilutions of parasitic tachyzoites. The results show that TaqMan probe-based real-time PRC is a sensitive and specific method for the detection of toxoplasmosis in immunocompromised individuals, as well as the LAMP assay, which can be used as a suitable alternative diagnostic method for the detection of toxoplasmosis in such patients, without need the for any expensive equipment.

Keywords LAMP · TaqMan probe-based real-time PCR · RE gene · Toxoplasmosis · Immunocompromised patients

Introduction

Toxoplasmosis infection is caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii* (*T. gondii*), which is one of the most common zoonotic infections between humans and animals. As an opportunistic parasite, *T. gondii* can be serious and sometimes fatal in high-risk groups, including immunocompromised patients (Burg et al. 1989). The average prevalence of toxoplasmosis in the world is

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estimated at 20–80% (Switaj et al. 2005), and 30–60% of the world's population are estimated to be infected by this parasite (Burg et al. 1989). Toxoplasmosis may be acquired by eating raw or under-cooked meat, containing parasitic tissue cysts or food or water contaminated with oocysts repelled by cats, blood and leukocyte transfusion, organ transplantation, or rarely by accidental inoculation in the laboratory, as well as through the placenta to the fetus (Dubey 2004; Lau et al. 2010). The clinical spectrum of *T. gondii* infection can be asymptomatic, or severe with serious pathologies in most infected individuals, affecting the CNS, eye, lymph node, heart and other organ involvement (Pujol-Riqué et al. 1999; Notomi et al. 2000; Dubey 2004; Kong et al. 2012). In immunocompetent hosts, the acute form of the disease recovers spontaneously without treatment, but in individuals with immune deficiency, including AIDS patients or people who take immunosuppressant drugs, there is a high likelihood of reactivation of brain cysts and the risk of host death. Also, *T. gondii* infection causes high mortality in infants with congenital toxoplasmosis (Homan et al. 2000; Karanis and Ongerth 2009; Rashno et al. 2016; Wang et al. 2017; Rostami et al. 2019, 2020). Therefore, the rapid and accurate diagnosis of toxoplasmosis plays an important role in the prevention and control of the disease, especially in those who are at risk.

Routine parasitological techniques such as the preparation of stained tissue sections and microscopic examination are time consuming and not very accurate (Badparva et al. 2009). Serologic methods including ELISA are the most common methods for the detection of toxoplasmosis, but these are not very sensitive in the diagnosis of infection during pregnancy or immunocompromised patients because specific antibodies to *Toxoplasma* appear late and their levels gradually increase (Fallahi et al. 2014; Liu et al. 2015; Hanifehpour et al. 2019). However, the PCR method that detects *Toxoplasma* DNA performs better than other current diagnostic methods, because of its sensitivity and specificity, as well as rapid PCR results, as it is independent of the immune status of the host (Karanis and Ongerth 2009; Zhang et al. 2009; Liu et al. 2015; Rostami et al. 2018). Optimization of PCR conditions should be done to prevent further amplification of unwanted nucleic acid otherwise the false sequence will be amplified. If such events occur in the early stages of this method, non-specific sequences will form a significant part of the final product (Homan et al. 2000; Krasteva et al. 2009). Therefore, such problems are prevented, using specific methods such as PCR product tracing that use specific real-time PCR probe, which increases the specificity of the technique, by applying a dedicated probe. In this method, the characteristics such as high sensitivity, which is 10–100 times higher, compared to agarose gel staining with ethidium bromide, no need for a dark room, ability to sample analysis on a large scale, automatic adjustment of the device, and no use of radioactive and carcinogenic substances such as ethidium bromide, as well as less contamination than methods such as Southern blot, have made this method so applicable with high safety and no health hazard (Fallahi et al. 2015, 2020).

Loop-mediated isothermal proliferation (LAMP) technique is one of the relatively novel nucleic acid amplification techniques that replicates the DNA with high specificity, efficiency, and speed, using four to six primers that identify six to eight specific regions of the template DNA and a sequence-specific DNA polymerase (*Bst DNA polymerase*) (Calderaro et al. 2006; Sotiriadou and Karanis 2008; Zhang et al. 2009; Fallahi et al. 2014, 2016, 2017). This method can reproduce up to 10^9 copies of the template in less than 1 h under isothermal conditions (63–67 °C). A simple incubator like a water bath or a heat block is sufficient for DNA amplification. The LAMP reaction products are detectable by electrophoresis, using ethidium bromide-stained agarose gel, through the observation of multiple bands with a ladder-shaped pattern, observation of green fluorescence due to the presence of SYBR Green I under UV light, and ocular observation of magnesium pyrophosphate white precipitate in reaction tubes (Sotiriadou and Karanis 2008; Krasteva et al. 2009; Zhang et al. 2009; Fallahi et al. 2015; Fallahi et al. 2018a, b). These features make it possible to use LAMP as a fast, accurate and simple test in the field conditions. Considering the clinical significance of *Toxoplasma* infection as an uncontrollable opportunistic infection in immunocompromised patients, and sometimes its potential fatal consequences in these patients, and disadvantages of routine toxoplasmosis detection by serology method such as ELISA, here, it was aimed to compare the LAMP and real-time PCR techniques based on the 529-bp repetitive element (RE) sequence in the diagnosis of toxoplasmosis in immunocompromised individuals with cancer and renal failure undergoing dialysis.

Materials and methods

Study population and sample collection

In this descriptive-analytic cross-sectional study, the study population included 119 immunocompromised patients (52 patients with renal failure undergoing dialysis and 67 cancer patients who were under chemotherapy treatment), in the oncology and hemodialysis sections of the Razi Educational Center, as well as the private center of the Kian Mehr Institute for Hemodialysis and Guilan Radiotherapy and Oncology Institute, Rasht, Iran. Ethical approval was received from the Ethics Committee of Guilan University of Medical Sciences (Ethical clearance for research was approved by the Medical Ethics Committee of Guilan University of Medical

Sciences) (IR.GUMS.REC.1396.406). At first, the purpose and importance of the research were described for all the participants, and then written consent was obtained from the patients who agreed to participate in the study. About, 2 ml of whole blood samples with EDTA were collected from each patient for DNA extraction. Samples were transferred to the Cellular and Molecular Research Center of Guilan University of Medical Sciences under the cold chain, and kept frozen at − 20 °C, until further use.

Molecular evaluation

The Rapid Genomic DNA Extraction (RGDE) kit was used for genomic DNA extraction from 119 whole blood samples by the salting-out method (Ali et al. 2008). For this, 500 µl of whole blood was transferred to a 1.5-ml microtube and 1 ml of cell lysis buffer was added. The mixture was shaken for 30 s and centrifuged at 4000×*g* for 2 min, and then the supernatant was discarded (this step was repeated twice). Subsequently, 400 µl nuclei lysis buffer, 120 µl saturated NaCl and 500 µl chloroform were added to the microtube and the mixture was centrifuged for 2 min at 4000×*g* (the microtube was shaken vigorously after adding each solution for 30 s). The top layer of the three formed layers was transferred to a new microtube; about 800 µl of ice-cold absolute ethanol was added and centrifuged for 2 min at 8000×*g*. Then the supernatant was discarded, the microtube was incubated at room temperature and left to dry. Finally, 20 µl distilled water was added to the tube and incubated at room temperature for 30 min. Ultimately, the microtube was kept at − 20 °C for later uses.

As a positive control, the *T. gondii* tachyzoites (RH strain) were obtained from the Department of Parasitology and Mycology of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The DNA extraction was done, using the DNG-Plus™ kit (Cinna Gen Inc., Tehran, Iran), according to the manufacturer's instructions. The quality and concentration of DNAs were determined by a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). The extracted DNAs were stored at − 20 °C, until further use. Moreover, double-distilled water (no DNA) was used as a negative control for each reaction.

RE-LAMP technique

The LAMP reaction was performed in a final volume of 25 µl, as previously described by Zhang et al. (2009). Primers used for the LAMP reaction based on the RE 529 bp sequence (Gene bank accession # AF146527) are shown in Table 1 (Zhang et al. 2009).

The ladder-like bands were visible by electrophoresis of the products and resolved on 1.5% agarose gel. Also, the positive tubes turned green by adding SYBR Green I to the reaction tubes after incubation at 63 °C, while the negative tubes remained orange. The magnesium pyrophosphate white precipitate, which is a by-product of the LAMP reaction, also helps to detect positive tubes.

TaqMan probe-based real-time PCR

Reaction was carried out in a final volume of 25 µl, containing 2.5 of µl template DNA (100 ng/µL), 200 nmol/L of each of forward and reverse primer, 100 nmol/L of probe, 12.5 µl of Real-Time Master mix (qPCR Probe Master, Jena Biosciences, Germany) and distilled water, using a real-time machine (Rotor-Gene 6000, Corbett Life Science).

The PCR condition was 2 min at 55 °C for enzyme activation, 10 min at 95 °C and 45 heat cycles, including 15-s denaturation at 95 °C, 15-s annealing at 62 °C and 20-s elongation at 68 °C. The beta-actin gene was used as the internal control for DNA isolation and replication in blood samples. In all reactions, the *T. gondii* RH strain was used as the positive control and double-distilled water as the negative control. The primers and probes for the real-time PCR based on the *Toxoplasma* RE gene are listed in Table 1 (Zhang et al. 2009).

Table 1 Summary of oligonucleotide primers/probe used in LAMP and real-time PCR for detection of *T. gondii* (12)

Parasite	Amplification system	Primer/probe	Sequences, 5′→3′
T. gondii	LAMP	BIP	TGGTTGGGAAGCGACGAGAGTTCCAGG AAAAGCAGCCAAG
		FIP′	
		LF	TCCTCACCCTCGCCTTCATCTAGGACTACA GACGCGATGC
		LB′	
		F3	TCCAAGACGGCTGGAGGAG
		B3	CGGAGAGGGAGAAGATGTTTCC
			CCACAGAAGGGACAGAAGTC
			TCCGGTGTCTCTTTTTCCAC
	Real-time PCR	ToxoRE_f	CAC AGA AGG GAC AGA AGT CGA A
		ToxoRE_r	CAG TCC TGA TAT CTC TCC TCC AAG A
		Probe ToxoRE_p	FAM-5′-CTA CAG ACG CGA TGC C-3′-BHQ1

The analytical sensitivity and specificity of LAMP and real-time PCR techniques

The analytical sensitivity of LAMP and real-time PCR techniques were evaluated by five-step serial dilutions of *T. gondii* tachyzoites from 0.25 to 5×10^5 spiked tachyzoites per milliliter of *Toxoplasma* seronegative blood sample. Genomic DNA of *Cryptosporidium spp.*, *Acanthamoeba spp.*, *Naegleria spp.*, *Leishmania spp.*, *Fasciola spp.,* and human chromosomal DNA were also used to determine the specificity of the LAMP and TaqMan probe-based real-time PCR techniques.

Analysis of the findings

After data collection, the Kappa coefficient test was used for data analysis, using SPSS 22 software, and the results were reported at the significance level of 0.05.

Results

RE-LAMP technique

The results of the LAMP technique showed that of the 119 samples, 5 samples (2.4%) were positive for *T. gondii* infection (Fig. 1). The analytical sensitivity of LAMP was determined, using serial dilutions of *T. gondii* tachyzoites in a negative serum blood sample of *T. gondii*, so that the limit of detection (LOD) of this technique was five *T. gondii* tachyzoites per milliliter of blood. Besides, none of the human chromosomal DNA samples and other parasites had a positive result in the LAMP technique (Fig. 2).

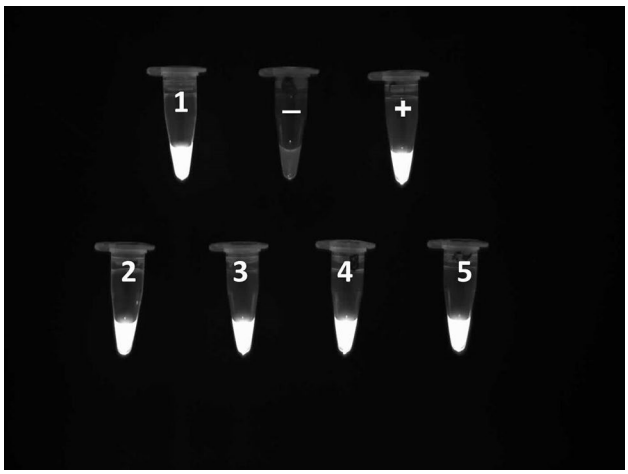


Fig. 1 The data analysis of patient samples, using visual inspection LAMP tubes in the gel doc: +, positive control; −, negative control; tubes 1–5, positive samples of patients with LAMP techniques

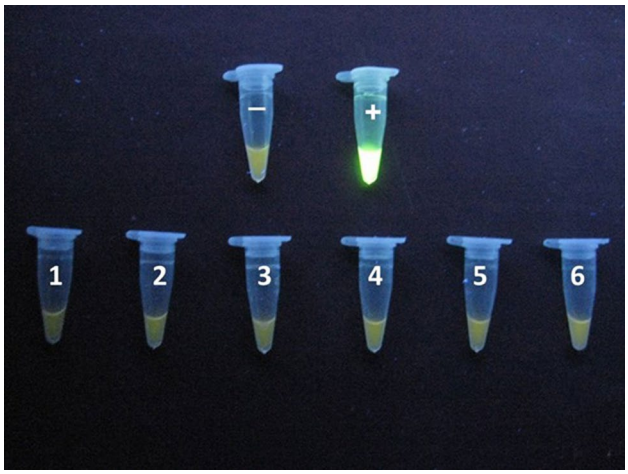


Fig. 2 Analytical characteristics of LAMP technique under UV light: +, positive control; −, negative control; tubes 1–6, genomic DNA of *Cryptosporidium*, *Acanthamoeba*, *Naegleria*, *Leishmania*, *Fasciola*, and human chromosomal DNA, respectively

TaqMan probe-based real-time PCR

The results of PCR showed that 7 (5.8%) of the 119 samples were positive for *T. gondii* infection. The LOD of TaqMan probe-based real-time PCR in negative serum samples was one *T. gondii* tachyzoite (CT: 38) per milliliter. Real-time PCR was 100% specific for the detection of *Toxoplasma,* and the positive results were achieved only with *T. gondii* DNA (Fig. 3).

Discussion

The rapid and accurate diagnosis of toxoplasmosis plays an important role in the prevention and control of the disease, especially in those who are at risk including immunocompromised individuals with cancer and renal failure undergoing dialysis. Nowadays various molecular methods including polymerase chain reaction (PCR) play an important role in the diagnosis of microbial infections such as toxoplasmosis. As reported in the literature reviews, the probe-based real-time PCR and LAMP techniques are among the most sensitive and specific diagnostic molecular assays for the detection of toxoplasmosis (Liu et al. 2015; Rostami et al. 2018).

In this study, after performing LAMP and TaqMan probe-based real-time PCR techniques based on the RE sequence, the LOD of RE-LAMP and real time-PCR methods were five and one *T. gondii* per milliliter, respectively, which demonstrates the higher sensitivity of the TaqMan probe-based real-time PCR technique in comparison with the LAMP assay. Both LAMP and real-time PCR methods were 100% specific, and the positive results were obtained only with *T. gondii* DNA, while other DNA samples were negative. In

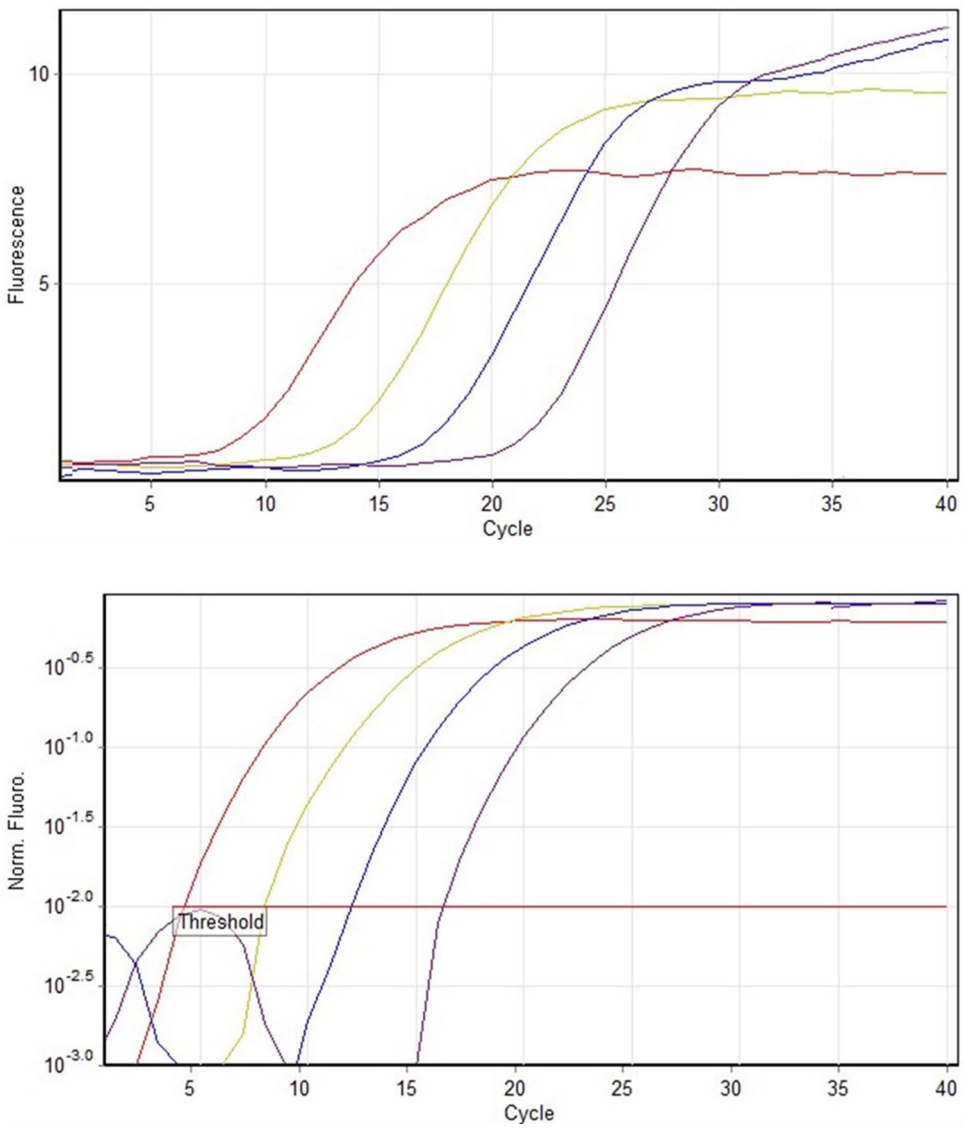
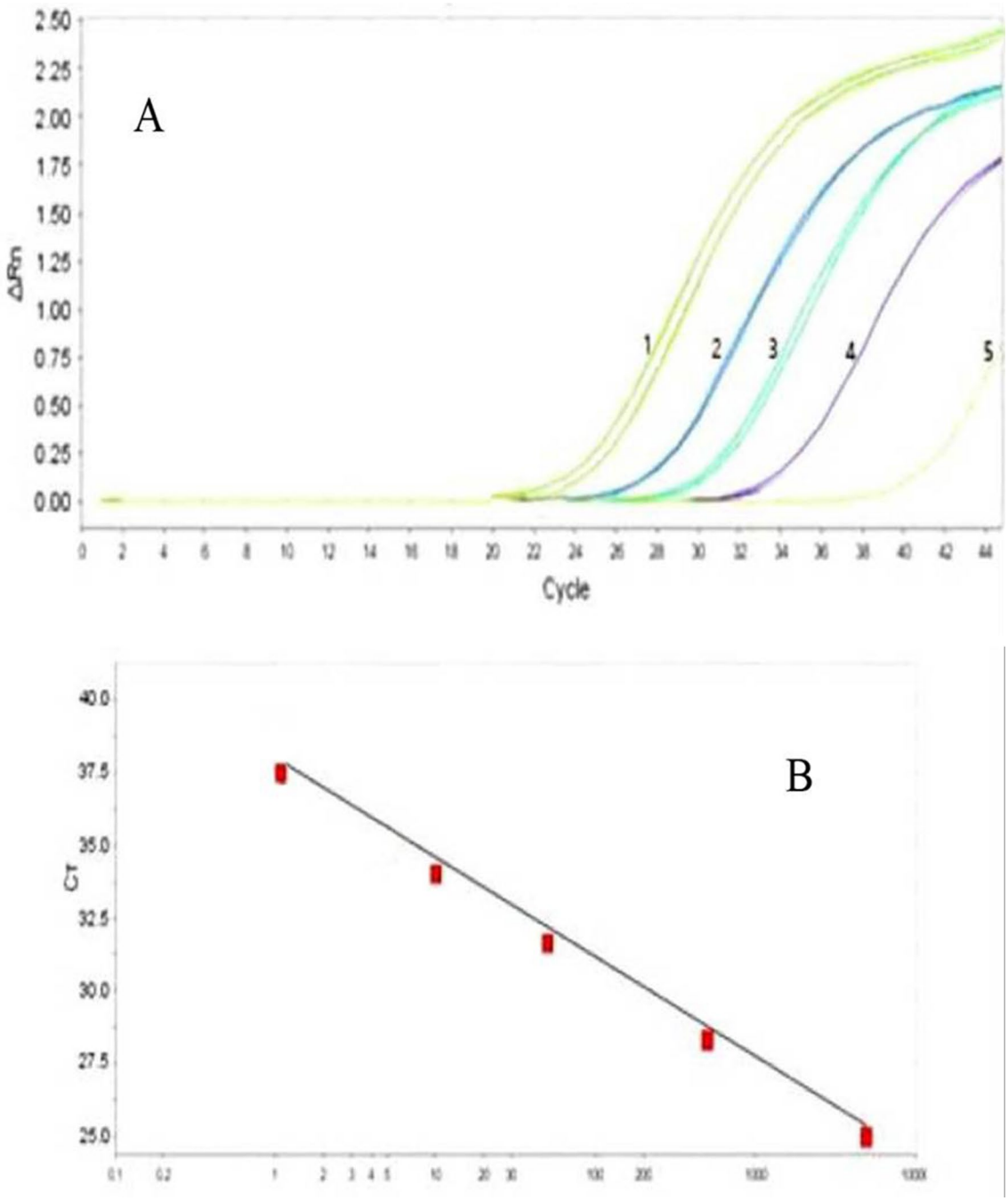


Fig. 3 Standard curve achieved from sequential dilution of *T. gondii* DNA. **a** Real-time CT values were plotted against five-step serial dilutions of *T. gondii* tachyzoites from 0.25 to 5×10^5 spiked tachyzoites per milliliter of *Toxoplasma* seronegative blood sample based on RE gene target. **b** Standard curve obtained from five-step serial dilutions of *T. gondii* tachyzoites in human seronegative blood sample

a study by Arefkhah and colleagues that was performed on sera and buffy coat of cancer and hemodialysis patients and healthy subjects, the seropositivity for the *Toxoplasma* IgM antibody was 2.1% in hemodialysis, 2% in cancer patients and 0.6% in healthy individuals. Only one cancer patient was positive for *Toxoplasma* DNA using PCR (Arefkhah et al. 2019). Compared to the results of the present study, the prevalence reported in cancer patients was lower in their study. Krasteva and colleagues used a standard PCR and LAMP technique, targeting the SAG1 gene to detect *T. gondii* in infected rat organs. Their results showed that the sensitivity of the LAMP assay was at least 1000 times higher than the standard PCR (Krasteva et al. 2009). As well, Lau et al. (2010) reported that the LAMP technique is ten times more sensitive than nested-PCR in the diagnosis of active toxoplasmosis in human blood samples. Contrary to the results of these studies, in our study, the sensitivity of the TaqMan probe-based real-time PCR assay was higher than the LAMP technique targeting the RE gene of *T. gondii*.

Calderaro et al. (2006) applied real-time PCR technique, using FRET and Taq-Man with 529 bp RE and 18 s rRNA, and nested-PCR technique with the B1 gene to detect toxoplasmosis in clinical samples. With a lower sensitivity compared to our results, their results indicated that the statistical sensitivity of Taq-Man and nested-PCR was 10^3, and for FRET method was 10^2 tachyzoites per milliliter (Calderaro et al. 2006). In a study by Lin et al. (2012), LAMP and real-time PCR methods were compared, using a 529 bp repetitive sequence in the detection of toxoplasmosis. With results almost similar to those of the present study, their results showed that the LOD of LAMP and real-time PCR were 10 and 1 fg of *T. gondii* DNA, respectively (Lin et al. 2012). In another study by Abdel Malek et al. in which the presence of anti-*Toxoplasma* antibodies in blood samples from 150

Fig. 3 (continued)



cancer patients and 50 control subjects have been examined using a lateral flow chromatographic immunoassay, the prevalence of anti-*T. gondii* antibodies was 20% for IgG and 4% for IgM in cancer patients, while in the control group, it was 8% and 2%, respectively. The difference was statistically significant for IgG but not for IgM (Abdel Malek et al. 2018). The results of this study are similar to those of our study, therefore, suggesting a high prevalence of toxoplasmosis in high-risk groups, including cancer patients.

According to the results, the present study showed that the sensitivity of the TaqMan probe-based real-time-PCR assay in the diagnosis of *T. gondii* is relatively higher than the LAMP method targeting the RE gene of *Toxoplasma*. Due to the high sensitivity and specificity of the TaqMan probe-based real-time PCR assay that targets the RE sequence, this assay is a useful tool in the diagnosis of *T. gondii*. Likewise, the RE-LAMP method has the potential to be an appropriate diagnostic method for the detection of toxoplasmosis in immunocompromised individuals. Also, the LAMP test can be carried out at 63 °C for 1 h, using a simple heating device, such as a water bath or a heat block, and on the other hand, DNA replication can be easily detected by observing the

turbidity, by the naked eye or the fluorescence of the reaction mixture. Therefore, this method can be used as a rapid and convenient molecular diagnostic tool in field tests, and even can be used in developing countries.

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References

Abdel Malek R, Wassef R, Rizk E, Sabry H, Tadros N, Boghdady A (2018) Toxoplasmosis an overlooked disease: seroprevalence in cancer patients. Asian Pac J Cancer Prev 19:1987–1991

Ali SM, Mahnaz S, Mahmood T (2008) Rapid genomic DNA extraction (RGDE). Forensic Sci Int Genet Suppl Ser 1:63–65

Arefkhah N, Hosseinib SA, Karimzadec R, Moshfec A, Hadiniad F, Abbasi Larkic R, Nazer Mozaffaric MA, Hadiniac A (2019) Seroprevalence and risk factors of *Toxoplasma gondii* infection among cancer and hemodialysis patients in southwest Iran. Clin Epidemiol Glob Health 7:596–599

Badparva E, Fallahi Sh, Sepahvand A, Pournia Y, Mollaei Rashnoo S (2009) The comparison of the efficacy of various fixatives on diverse staining methods of *Giardia lamblia* cyst. Pak J Biol Sci 12:1212–1216

Burg JL, Grover CM, Pouletty P, Boothroyd J (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol 27:1787–1792

Calderaro A, Piccolo G, Gorrini C (2006) Comparison between two real-time PCR assays and a nested-PCR for the detection of *Toxoplasma gondii*. Acta Bio Medica Atenei Parmensis 77:75–80

Dubey J (2004) Toxoplasmosis—a waterborne zoonosis. Vet Parasitol 126:57–72

Fallahi Sh, Seyyed Tabaei SJ, Pournia Y, Zebardast N, Kazemi B (2014) Comparison of loop-mediated isothermal amplification (LAMP) and nested-PCR assay targeting the RE and B1 gene for detection of *Toxoplasma gondii* in blood samples of children with leukaemia. Diag Microbiol Infect Dis 79:347–354

Fallahi Sh, Arab-Mazar Z, Ghasemian M, Haghighi A (2015) Challenging loop-mediated isothermal amplification (LAMP) technique for molecular detection of *Toxoplasma gondii*. Asian Pac J Trop Med 8:366–372

Fallahi Sh, Rostami A, Mohammadi M, Ebrahimzadeh F, Pournia Y (2016) Practical parasitology courses and infection with intestinal parasites in students. J Infect Public Health 9:654–660

Fallahi Sh, Rostami A, Birjandi M, Zebardast N, Kheirandish F, Spotin A (2017) Parkinson's disease and *Toxoplasma gondii* infection: sero-molecular assess the possible link among patients. Acta Trop 173:97–101

Fallahi Sh, Rostami A, Nourollahpour Shiadeh M, Behniafar H, Paktinat S (2018a) An updated literature review on maternal-fetal and reproductive disorders of *Toxoplasma gondii* infection. J Gynecol Obstet Hum Reprod 47:133–140

Fallahi Sh, Moosavi SF, Karimi A, Chegeni AS, Saki M, Namdari P, Rashno MM, Varzi AM, Tarrahi MJ, Almasian M (2018b) An advanced uracil DNA glycosylase-supplemented loop-mediated isothermal amplification (UDG-LAMP) technique used in the sensitive and specific detection of *Cryptosporidium parvum*, *Cryptosporidium hominis*, and *Cryptosporidium meleagridis* in AIDS patients. Diag Microbiol Infect Dis 91:6–12

Fallahi S, Babaei M, Rostami A, Mirahmadi H, Arab-Mazar Z, Sepahvand A (2020) Diagnosis of Candida albicans: conventional diagnostic methods compared to the loop-mediated isothermal amplification (LAMP) assay. Arch Microbiol 202(2):275–282

Hanifehpour H, Samsam Shariar SK, Ghafari MS, Kheirandish F, Saber V, Fallahi SH (2019) Serological and molecular diagnosis of *Toxoplasma gondii* Infections in thalassemia patients. Iran J Parasitol 14:20–28

Homan W, Vercammen M, De Braekeleer J, Verschueren H (2000) 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 30:69–75

Karanis P, Ongerth J (2009) LAMP—a powerful and flexible tool for monitoring microbial pathogens. Update 25:498–499

Kong QM, Lu SH, Tong QB, Lou D, Chen R, Zheng B, Kumagai T, Wen LY, Ohta N, Zhou XN (2012) Loop-mediated isothermal amplification (LAMP): early detection of *Toxoplasma gondii* infection in mice. Parasites Vectors 5:2

Krasteva DM, Toubiana S, Hartati A, Kusumawati J, Dubremetz J, Widada S (2009) Development of loop-mediated isothermal amplification (LAMP) as a diagnostic tool of toxoplasmosis. Vet Parasitol 162:327–331

Lau YL, Meganathan P, Sonaimuthu P, Thiruvengadam G, Nissapatorn V, Chen Y (2010) Specific, sensitive, and rapid diagnosis of active toxoplasmosis by a loop-mediated isothermal amplification method using blood samples from patients. J Clin Microbiol 48:3698–3702

Lin Z, Zhang Y, Zhang H, Zhou Y, Cao J, Zhou J (2012) Comparison of loop-mediated isothermal amplification (LAMP) and real-time PCR method targeting a 529-bp repeat element for diagnosis of toxoplasmosis. Vet Parasitol 185:296–300

Liu Q, Wang ZD, Huang SY, Zhu XQ (2015) Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasites Vectors 8:1

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:E63

Pujol-Riqué M, Derouin F, Garcia-Quintanilla A, Valls M, Miro J, De Anta MJ (1999) Design of a one-tube hemi-nested PCR for detection of *Toxoplasma gondii* and comparison of three DNA purification methods. J Med Microbiol 48:857–862

Rashno MM, Fallahi S, Kheirandish F, Bagheri S, Kayedi MH, Birjandi M (2016) Seroprevalence of *Toxoplasma gondii* infection in patients with Alzheimer's disease. Arch Clin Infect Dis 11(3):e37205

Rostami A, Karanis P, Fallahi Sh (2018) Advances in serological, imaging techniques and molecular diagnosis of *Toxoplasma gondii* infection. Infect 46:303–315

Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, Foroutan M, Behniafar H, Taghipour A, Maldonado YA, Mokdad AH, Gasser RB (2019) Acute *Toxoplasma* infection in pregnant women worldwide: a systematic review and meta-analysis. PLoS Negl Trop Dis 13:e0007807

Rostami A, Riahi SM, Gamble HR, Fakhri Y, Nourollahpour Shiadeh M, Danesh M, Behniafar H, Paktinat S, Foroutan M, Mokdad AH, Hotez PJ, Gasser RB (2020) Global prevalence of latent toxoplasmosis in pregnant women: a systematic review and meta-analysis. Clin Microbiol Infect **(Article In Press)**

Sotiriadou I, Karanis P (2008) Evaluation of loop-mediated isothermal amplification for detection of *Toxoplasma gondii* in water samples and comparative findings by polymerase chain reaction and immunofluorescence test (IFT). Diag Microbiol Infect Dis 62:357–365

Switaj K, Master A, Skrzypczak M, Zaborowski P (2005) Recent trends in molecular diagnostics for *Toxoplasma gondii* infections. Clin Microbiol Infect 11:170–176

Wang ZD, Wang SC, Liu HH, Ma HY, Li ZY, Wei F, Zhu XQ, Liu Q (2017) Prevalence and burden of *Toxoplasma gondii* infection

in HIV-infected people: a systematic review and meta-analysis. Lancet HIV 4:e177–e188

Zhang H, Thekisoe OM, Aboge GO, Kyan H, Yamagishi J, Inoue N, Nishikawa Y, Zakimi S, Xuan X (2009) *Toxoplasma gondii*: sensitive and rapid detection of infection by loop-mediated isothermal amplification (LAMP) method. Exp Parasitol 122:47–50

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