Serological and molecular rapid diagnostic tests for *Toxoplasma* infection in humans and animals

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Abstract

Infection by *Toxoplasma gondii* is prevalent worldwide. The parasite can infect a broad spectrum of vertebrate hosts, but infection of fetuses and immunocompromised patients is of particular concern. Easy-to-perform, robust, and highly sensitive and specific methods to detect *Toxoplasma* infection are important for the treatment and management of patients. Rapid diagnostic methods that do not sacrifice the accuracy of the assay and give reproducible results in a short time are highly desirable. In this context, rapid diagnostic tests (RDTs), especially with point-of-care (POC) features, are promising diagnostic methods in clinical microbiology laboratories, especially in areas with minimal laboratory facilities. More advanced methods using microfluidics and sensor technology will be the future trend. In this review, we discuss serological and molecular-based rapid diagnostic tests for detecting *Toxoplasma* infection in humans as well as animals.

Keywords *Toxoplasma* infection · *Toxoplasma gondii* · Rapid diagnostic tests · Immunoassay · Molecular diagnostics · Point-of-care test

Introduction

Congenital *Toxoplasma* infection is the transmission of *T. gondii* from mother to fetus through the placenta, leading to potential fetal death, abortion, infantile neurologic defects, and chorioretinitis [1–3]. The incidence of congenital *Toxoplasma* infection varies from region to region; however, the prevalence is proportional to the socioeconomic conditions, unhygienic lifestyle, and feline population [3, 4]. Early detection of congenital *Toxoplasma* infection may reduce transplacental transmission and lessen the fetal disease burden [5, 6]. Usually, *T. gondii* infection in pregnant women is asymptomatic, and countries like Austria [7], France [8], and Brazil [9] conduct routine maternal screening to identify the infected pregnant woman and provide early treatment.

*T. gondii* remains dormant in infected tissues and may reactivate in immunocompromised individuals, leading to potentially fatal complications [10, 11]. HIV patients and hematopoietic stem cell and heart transplant recipients are more susceptible to *T. gondii* infection [12]. Cerebral *Toxoplasma* infection is considered the third most prevalent opportunistic illness in HIV-infected populations [13].

In veterinary medicine, *Toxoplasma* infection is also a serious health concern. *T. gondii* infects a vast range of animal species, including wild, pet, and domesticated animals. *T. gondii* oocysts in feline feces contaminate the surroundings and undergo sporulation in terrestrial and aquatic environments. The oocysts are resistant to various chemicals and disinfectants that are routinely used by the water supply industry; thus, contaminated water reservoirs are a significant factor in outbreaks of *Toxoplasma* infection in humans and animals [14–16]. In small ruminants, the infection may lead to abortions, fetal mummification, stillbirths, or births of weak offspring [17, 18]. As compared to its prevalence in cattle, the prevalence of *T. gondii* is higher in sheep, goats, and pigs [19, 20]. *T. gondii* was detected mainly in sheep’s brain and heart tissues; in contrast, no parasite was detected in tissue samples taken from experimentally infected cattle [21]. Reproductive failure in goats and sheep is a substantial economic loss, which may impact the lives of people who are dependent on ruminant meat production as a source of income.

In developed countries and big cities in developing countries, medical laboratories favor the use of fully automated systems such as BioPlex™ 2200 (Bio-Rad) that ensure quality...
and allow high-throughput testing [22]. However, most of the tests are neither accessible nor affordable to patients in the developing world. A point-of-care (POC) test is a rapid diagnostic test (RDT), providing test results to the patients at health centers, in the field, or at screening sites. RDT is considered advantageous over central laboratory testing in terms of robustness, simplicity, and ease in handling [23]. According to the World Health Organization, an RDT device should comply with the guidelines termed as “ASSURED” which is an acronym for affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free, and deliverable to those who need it [24–26]. Although RDT devices for HIV infection, malaria, and syphilis are available [27], efforts are needed to develop RDT for a broad range of infections to reduce the mortality and morbidity cases in remote areas. While devising an RDT, several variables including the characteristic features of the infected population, antigen, genetic variation of the pathogen and/or host, the test methodology (use of recombinant or native antigen), and the physical format of the test (manual or automated) must be considered and optimized [28]. The present review discusses RDTs based on immunoassays and molecular methods for detecting T. gondii infection in both humans and animals.

Rapid immunodiagnostic tests

A broad range of serological tests for Toxoplasma infection is available with various methodologies and antigens which can detect different immunoglobulin isotypes. Most are not RDTs and are targeted for laboratories with good infrastructure and skilled personnel. The assays include Sabin-Feldman dye test (DT), enzyme-linked immunosorbent assay (ELISA), immunosorbent agglutination assay (ISAGA), indirect hemagglutination assay (IHA), indirect fluorescent antibody tests (IFATs), modified agglutination test (MAT), and Western blot (WB) [29].

Lateral flow assay

In the lateral flow assay (LFT), a sample buffer is used to drive the test sample (e.g., serum or blood) along a solid substrate through capillary action, and the result is obtained in less than 30 min [30]. This assay, also called immunochromatography test (ICT), has become a well-established and widely accepted POC approach [31, 32]. As compared to other serum-based diagnostic tests, ICT is simple, rapid, and cost-effective and demonstrates high sensitivity and specificity, making it suitable for field application [33]. Table 1 shows RDTs that have been reported for detection of Toxoplasma infection in humans and animals. Toxoplasma ICT IgG-IgM (LDBIO Diagnostic, Lyon, France; LDBIO) is a commercial rapid POC test, which detects Toxoplasma-specific IgG/IgM antibodies. T. gondii native antigen used as the test line and rabbit gamma globulin as the control line are blotted on a nitrocellulose membrane. At one end of the cassette, red latex particles conjugated with T. gondii antigen and blue latex particles conjugated with goat anti-rabbit antibody are adsorbed on fiberglass support. After dispensing the serum sample, if specific IgG/IgM is present, a red line will appear at the test line while a blue line will appear at the control line [41]. This POC test is highly effective and can be considered a first-line screening test. The device accurately identified seropositive USA patients with known T. gondii infection and distinguished them from seronegative individuals. Moreover, it has the potential to identify acute infection accurately [34, 41]. In the USA, both the diagnostic sensitivity and specificity were found to be 100% [34], while in France, the sensitivity and specificity were reported to be 97% and 96%, respectively, as compared to the Architect fully automated chemiluminescence test (Abbott North, Chicago, IL) [41].

The diagnostic values of the LDBIO test and two other commercial POC tests, i.e., Toxo IgG/IgM Rapid test (Biopanda Reagents, Belfast, UK) and OnSite Toxo IgG/IgM Combo Rapid Test (CTK-Biotech, San Diego, CA), were compared. They were evaluated against reference tests used at the Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL) [35]. The sensitivity (n = 170) of all the three tests for T. gondii-specific IgG detection was 100% while the specificity (n = 80) was almost similar across the tests, i.e., Biopanda 96.3%, OnSite 97.5%, and LDBIO 98.8%. In contrast, the sensitivity (n = 82) of the POC tests for IgM detection was variable, i.e., Biopanda 62.2%, OnSite 28%, and LDBIO 100% [35]. The Biopanda and OnSite devices also displayed poor visualization of test lines due to the faint color in a proportion of the tests performed. Since the LDBIO test demonstrated 100% sensitivity and exhibited negligible false-positive results, it is advantageous for universal screening of Toxoplasma infection [35]. In short, the LDBIO POC test addresses the limitations of automated screening methods, i.e., not sufficiently sensitive for IgG detection and lack of specificity for IgM detection [42].

The LFT cassette test architecture may also influence the test sensitivity. The LDBIO test detects both IgG and IgM antibodies using a nitrocellulose membrane strip lined with native Toxoplasma antigen and latex-coated Toxoplasma antigen adsorbed on the conjugate pad. Thus, anti-human IgG/IgM antibody is not used as a test reagent. On the other hand, the Biopanda and OnSite tests have two distinct lines for IgG and IgM detection. In both tests, anti-human IgG and IgM antibodies are separately lined on the nitrocellulose strip; however, the Biopanda test uses two nitrocellulose strips, one for each antibody class, while in the OnSite test, the two antibodies are lined separately on one nitrocellulose strip. Both Biopanda and OnSite tests use colloidal gold-conjugated recombinant protein adsorbed on the conjugate pad. All three tests also include a control line.
Table 1 Immunochromatography (ICT) tests that detect Toxoplasma infection in humans and animals

| Test name                  | Component being detected | Antigen/antibody used in test | Reference test          | Sample size and type | Sensitivity | Specificity | References |
|----------------------------|--------------------------|-------------------------------|-------------------------|---------------------|-------------|-------------|------------|
| *LDBIO Toxoplasma ICT IgG-IgM | IgG and IgM              | Whole-cell Toxoplasma lysate  | Sabin-Feldman dye test and IgM-ELISA | Human              | 100%        | 100%        | [34]       |
| Biopanda Toxo IgG/IgM      | IgG/IgM                  | Recombinant T. gondii antigen | Sabin-Feldman-IgG dye test and IgM-ELISA | 310 Human         | 100% (IgG)  | 96.3% (IgG) | [35]       |
| OnSite Toxo IgG/IgM        | IgG/IgM                  | Recombinant T. gondii antigen | Sabin-Feldman-IgG dye test and IgM-ELISA | 310 Human         | 62.2% (IgM) | 88.5% (IgM) | [35]       |
| Lab-based test IgG         | IgG                      | N-terminal rSAG1A linked to GRA2 | ELISA                   | 67 Human           | 97.1%       | 100%        | [36]       |
| Lab-based test IgG         | IgG                      | rSAG2                         | LAT and ELISA           | 179 Cats           | 97.2% (IgM) | 95.8% (ELISA) | [37]       |
| Lab-based test Circulating antigen | Anti-ESA IgG | Anti-ESA IgG                  | ELISA                   | 80 Pigs            | 98%         | 100%        | [38]       |
| Lab-based test IgG         | IgG                      | SAG3                          | ELISA                   | 310 Pigs           | 100%        | 99.65%      | [39]       |
| Lab-based test IgG         | IgG                      | rSAG1                         | ELISA                   | 182 Cats           | 100%        | 99.4%       | [40]       |

*Commercialized test: Toxoplasma ICT IgG-IgM (LDBIO Diagnostic, Lyon, France), Toxo IgG/IgM rapid test (Biopanda Reagents, Belfast, UK), OnSite Toxo IgG/IgM Combo Rapid Test (CTK-Biotech, San Diego, CA)

The use of recombinant antigens has the advantage of standardized antigen production. In the past two decades, several Toxoplasma recombinant antigens have been cloned and expressed in E. coli or yeast for use as diagnostic reagents for human and animal infections. They include antigens from the parasite surface (SAGs), matrix (MAGs), dense granule (GRAs), rhoptry (ROPs), and microneme (MICs) [43–50]. Four of the recombinant antigens (SAG1, SAG2, GRA3, and GRA6) expressed in E. coli have been used in an RDT to detect infection in cats. Among them, recombinant SAG1 demonstrated the highest sensitivity and specificity, i.e., 100% and 99.2%, respectively [40]. In another study, the N-terminal half of SAG1A was linked to the intrinsic unstructured domain of GRA2 and conjugated with 40-nm colloidal gold particles [36]. The structurally modified rSAG1A-GRA2-based RDT showed 100% specificity and 97.1% sensitivity for detection of human Toxoplasma infection as compared to a laboratory ELISA that used T. gondii whole cell lysate antigen [36]. An RDT kit comprising GST-linked SAG1A has been used to detect Toxoplasma infection in Seokmo-do (Island) in Ganghwa-gun, Incheon City, South Korea [51].

The diagnostic value of gold colloid-conjugated recombinant GRA7 for rapid detection of Toxoplasma infection has been evaluated in animals [52]. The ICT identified T. gondii-infected mice from those unaffected or infected with Neospora caninum. Pig sera were also tested with the ICT, and the results were found to be quite consistent with those obtained using the latex agglutination test (LAT) and indirect ELISA. However, samples with low OD (< 0.3) values in GRA7-based ELISA were negative in the ICT since the low titer of antibody in the samples could not capture enough conjugated antigen to develop a color reaction on the strip [52]. Nevertheless, the ICT using GRA7 demonstrated promising results as a diagnostic test for routine rapid testing in the clinic and mass screening of animal samples in the field. In a more recent study, ICT strips based on GRA7 have been used to detect Toxoplasma-specific serum IgG in human samples [53]. The diagnostic sensitivity of the assay was 100% while the specificity was 96.7%, with only one false-positive result.

Wang et al. used ICT strips to detect Toxoplasma circulating antigens in the blood of animals that were experimentally infected with the Gansu Jingtai strain of T. gondii [38]. Immunoglobulin G, specific to excretory/secretory antigens (ESA) of T. gondii, was produced in sheep immunized intramuscularly with the antigen. Purified anti-ESA IgG was conjugated with gold, and the resulting colloidal gold-conjugated anti-ESA IgG was blotted on a glass fiber. Sera from experimentally infected animals including pigs, goats, sheep, and rabbits that were intraperitoneally infected with tachyzoites were analyzed, and the results were compared with ELISA. A 100% agreement between both assays was reported; moreover, the strips did not show any cross-reactivity with antigens of Neospora caninum [38]. In a more recent study, an ICT comprising gold particles conjugated with a monoclonal antibody against SAG3, which is expressed in all the infective stages of T. gondii, was analyzed [39]. The assay demonstrated higher sensitivity and specificity compared to another antibody assay which used GRA7 recombinant antigen [52].
The detection limit of the SAG3-ICT was 100 ng with visual detection under optimal conditions of analysis. No cross-reactivity was found when evaluated with porcine serum samples infected with Cryptosporidium suis, Mycoplasma suis, Streptococcus suis, Salmonella cholerae suis, Cysticercus cellulosae, Isospora suis, and Trichinella spiralis.

For detection of Toxoplasma infection in cats and dogs, a modified version of ICT assay, the dynamic flow immunochromatographic test (DFICT) which utilizes immunochromatography and fluid dynamics, has been proposed [54]. T. gondii recombinant antigen and staphylococcal protein A (SPA), fixed on nitrocellulose membrane at points designated as a test (T) and control (C), respectively, were used as capture reagents. A volume of 100 μl liquid gold-SPA conjugate was added to the reagent hole, and 5 μl of serum was added to the sample hole, and the result was visualized within 5 min. The assay was highly sensitive and detected antibodies even when at 1:320 serum dilution, and demonstrated no cross-reactivity with antibodies from other related canine pathogens (distemper virus, parvovirus, coronavirus, leishmania, and Neospora caninum) or feline pathogens (panleukopenia virus, calicivirus, and N. caninum). Furthermore, the DFICT did not show any change in sensitivity or specificity when stored at 4 °C for several months. A high degree of consistency was seen between DFICT and a commercial ELISA kit, suggesting that the novel test strip was reliable [54]. The conjugate pad in an ICT is dried gold-conjugate on glass fiber membranes, whereas the DFICT makes use of liquid gold conjugate, thereby making the manufacturing process for DFICT simpler since no drying facility or low humidity control is required. Also, in an ICT assay, the serum sample is driven by a chase buffer [31, 55], while in a DFICT, there is no need for washing or blocking during sample addition, between sample and visualization, or at the termination step of the reaction [54].

A fluid-phase immunoassay overcomes some disadvantages of a solid-phase immunoassay such as suboptimal detection of conformational epitopes, high background signal, and narrow dynamic range of detection, which may sometimes lead to false-positive results and lower sensitivity [56, 57]. An example of a fluid-phase immunoassay is luciferase immunoprecipitation system (LIPS) which makes use of light-emitting luciferase-recombinant antigen fusion protein to quantitatively determine serum antibody titers [57]. The highly linear light output of the luciferase increases the dynamic range of detection to about seven orders of magnitude. LIPS has the potential to detect antibodies associated with various autoimmune and infectious diseases, and is advantageous in terms of short incubation time, little background signal, high sensitivity and specificity, and having the ability to be multiplexed [57–59]. A LIPS-based rapid immunoassay, LIPSTICKS, has been designed for rapid detection of serum antibodies in one minute [60]. Fast reaction kinetics, the ability to efficiently detect conformational epitopes, quantitative results, and a large dynamic range of detection make LIPSTICKS superior to ICT. Additionally, LIPSTICKS requires a very small volume of serum (0.1 μl) or saliva (10 μl) per test and a simple dilution step [60]. LIPSTICKS can be developed for a wide range of diseases by merely changing the luciferase-tagged antigen [60]. LIPS technology has been successfully employed to diagnose a wide range of diseases including hepatitis C virus (HCV) [61], Lyme disease [62], strongyloidiasis [63], loaisis [64], and onchocerciasis [65]. More recently, Aye et al. [66] have used LIPS for the serodiagnosis of Toxoplasma infection. Four fusion proteins of nanoluminescent (Nluc, a small luciferase enzyme) and T. gondii antigens including GRA6, GRA7, GRA8, and bradyzoite antigens (BAG1) expressed in E. coli were used in the assay. With sera from experimentally infected mice, LIPS assay detected Toxoplasma-specific antibodies against Nluc-GRA6, Nluc-GRA7, and Nluc-GRA8 as early as day 14. Meanwhile, the antibody to Nluc-BAG1 was not detected even up to day 21; however, the antibody appeared in the serum on day 60. The detection limits of the LIPS assay with standard human sera (WHO standard anti-Toxoplasma human immunoglobulin, TOXM) were 3.9, 2, 1, and 1 IU/ml for rGRA6, rGRA7, rGRA8, and rBAG1, respectively [66].

**Immunosensors**

Biosensors are diagnostic devices that change biological responses to a quantifiable signal. These devices have two main components, a bioreceptor for target recognition and a transducer which converts this recognition into an electrical signal [67]. Based on signal transduction methods, immunosensors can be divided into four main groups, i.e., electrochemical, optical, piezoelectric, and thermal or calorimetric [30, 68]. Chip-based miniaturized, portable, and self-containing immunosensors are highly desired in diagnostic laboratories for detecting serum biomarkers. Advances in nanotechnology have led to the development of nanomaterials including gold nanoparticles, carbon nanotubes, magnetic nanoparticles, and graphene that could be used in immunosensors [69].

**Optical and piezoelectric biosensors**

Optical detection of fluorescent labels or apparent color change due to enzymatic reaction is the most common detection method and regarded as the “gold standard.” These methods have high detection sensitivities ranging from nanogram to picogram per milliliter and are the simplest forms of biosensor signals to generate and analyze [70, 71]. The generated optical signal is either fluorescent or luminescent; the former is a result of excitation of a label such as a fluorophore or quantum dot immobilized on the surface of the sensor. A luminescent signal is produced after an
enzymatic reaction of horseradish peroxidase with a luminogenic substrate such as luminol and luciferin.

Surface plasmon resonance is an optical phenomenon based on the generation of electromagnetic waves (plasmons). In a plasmonic immunosensor, a metallic surface is prepared by immobilizing specific antibodies. Antigen/antibody interaction on the metallic surface changes the refractive index/thickness, resulting in a shift in the resonance curve of the reflected light that is directly related to the concentration of the bound biomarker [72]. High sensitivity and broad dynamic range make the plasmonic biosensor ideal for clinical diagnostic applications [73]. The plasmonic gold film can detect a panel of antibodies over an array of spatially defined antigen spots, which gives a multicolor fluorescence in the visible-to-near infrared region (500 to 900 nm). The test can be performed with a single drop of serum or whole blood. The multicolor detection scheme has the potential to differentiate between patients with acute and chronic infections. The sensitivity and specificity of the method were reported to be similar to those of well-established assays used in the USA National Reference Laboratories for *Toxoplasma* infection [74]. The potential of the plasmonic gold chips to detect *Toxoplasma* IgG was similar to that of the IgG dye test [75, 76]. Also, the plasmonic gold chip can detect IgG, IgM, and IgA antibodies in serum or whole blood in a single run [74] and is comparable to the commercial multiplexed test using the BioPlex 2200 system (Bio-Rad) which is based on Luminex technology [22]. The approximate time for the detection of IgG, IgM, and IgA antibodies with a plasmonic gold chip is about 2 h at USD10 per patient [74]. With ~1 μl serum sample, the multiplex plasmonic gold platform has been used for the detection of IgG/IgM in seroconverted, chronically infected, non-infected, and newborns in Nice, France [77]. Other advantages of the plasmonic biosensor are its potential to be miniaturized and multiplexed. By integrating plasmonic biosensors with microfluidics, a rapid POC device can be developed [78]. Economical fabrication methods to build these nanostructures have been developed by using plastics and polymers as substrates [79, 80].

Laser-induced fluorescence (LIF) is another type of optical detection approach, which is broadly used for the microfluidic immunosensor design [81, 82]. Recently, LIF microfluidic chips have been used in many research fields including lab-on-chip [83], POC testing [84, 85], and organic compound analysis [86]. A microfluidic immunosensor based on LIF has been developed for the quantitative detection of IgG antibodies against *T. gondii* in humans [87]. Zinc oxide nanoparticle covered with chitosan was used to conjugate *T. gondii* antigen and placed into a central microfluidic channel. Serum samples from patients with *Toxoplasma* infection were incubated with *T. gondii* antigen on the microfluidic channel. The signal was generated by adding alkaline phosphatase (ALP)-labeled anti-IgG antibody, followed by the substrate, non-fluorescent 4-methylumbelliferyl phosphatase, which is converted to fluorescent product. The relative fluorescence of the enzymatic product was quantified, and the intensity of the fluorescence was directly linked to the concentration of serum antibodies [87]. The incorporation of chitosan-coated zinc oxide nanoparticles into the microfluidic channel improved the sensitivity as well as simplified the operating procedure by reducing the analysis time to 31 min [87]. Attempts have been made to exploit the potential POC benefits of microfluidics by miniaturizing and integrating the optical elements, including using amorphous silicon photodiodes [88] and optical fiber light guides [89]. A POC device based on LIF microfluidics has the potential to decrease the time and quantity of reactants for analysis, along with multiplexing and portability [84].

Aptamers are single-stranded nucleic acid molecules that specifically bind to the target molecules and are widely used in diagnosis, as target delivery agents, and for therapeutic purposes [90]. The process of selecting aptamers is called systematic evolution of ligands by exponential enrichment (SELEX). Structure versatility, high affinity to their targets, and easy conjugation, as well as easy labeling, are some of the attractive features of aptamers for use in developing diagnostic tools [91]. A quantum dots-based aptasensor for the detection of *Toxoplasma* infection in humans has been reported [92]. As compared to conventional ELISA and colloidal gold immunodot assay, the aptasensor (aptamer-IgG-aptamer complex) showed increased sensitivity [92].

Piezoelectric biosensors, based on quartz chips, are promising transducers for the rapid and simple detection of viruses, bacteria, proteins, nucleic acids, and small molecules [93]. Piezoelectric immunosensors are mass-sensitive devices in which the agglutination of an immobilized antigen/antibody on the surface of an oscillating quartz and target biomarker antigen/antibody increases the mass of the crystal and proportionally reduces the frequency of oscillation of the crystals. The immunosensor then records and quantifies the variation in the frequency of the electrical signal produced due to the antigen-antibody agglutination. A gold nanoparticle-based piezoelectric immunosensor has been used to detect *T. gondii*-specific antibodies in serum of infected rabbits [94]. The immunosensor was highly sensitive and demonstrated significant results even in the presence of highly diluted (1:5500) anti-*T. gondii* antibody [94]. Moreover, the analytical results were consistent with that of ELISA; thus, this technique could be a promising method for diagnosing *Toxoplasma* infection [94].

Electrochemical biosensors

An electrochemical biosensor utilizes the electrocatalytic activity of graphene sheets, a tightly packed flat layer of carbon atoms which act as a two-dimensional material. It has unique physicochemical properties like rapid electron transport, excellent electrical conductivity, and thermal stability [95, 96].
Good performance of electrochemical biosensors has been achieved by improving gold-coated magnetic nanoparticles in terms of their morphology, particle size, effective surface area, functionality, adsorption capacity, and electron transfer properties [97, 98]. A sandwich-type electrochemical biosensor with anti-IgM goldmag and graphene sheets has been proposed for detecting T. gondii-specific IgM antibodies [99]. The use of goldmag is a simple method which does not need chemicals for immunosensor regeneration. T. gondii antigen was immobilized onto goldmag nanoparticles attached to a nafion-graphene sheets-modified electrode. Following incubation with serum and HRP-labeled anti-IgM antibodies, an electrochemical signal was generated by adding hydrogen peroxide as the substrate [99]. The biosensor was biocompatible and demonstrated good conductivity and specificity.

**Molecular diagnostics**

Direct detection of acute Toxoplasma infection in humans can be performed by polymerase chain reaction (PCR), which amplifies the DNA of T. gondii in amniotic fluid, eye fluid, tissues, or blood [100, 101]. For the rapid and sensitive detection of Toxoplasma infection, real-time PCR assays and targets including B1 gene which occurs in 35 copies in T. gondii genome have been reported [102–104]. A 529-bp (GenBank Accession No. AF146527) sequence of unknown function which is repeated 200- to 300-fold in T. gondii genome was used to develop a sensitive and specific PCR assay [105]. This sequence was found to be present in all the 60 strains of T. gondii tested, and distinguished the DNA of T. gondii from the DNA of humans and mice, or DNA from other parasites, including Echinococcus granulosis, Giardia duodenalis, Plasmodium falciparum, Sarcocystis spp., Trichinella spiralis, Trichomonas vaginalis, and Neospora caninum [105]. A comparative study of the amplification of the 529-bp sequence and B1 gene using a low amount of DNA has shown that detection of the former sequence increased the diagnostic sensitivity and accuracy of the assay [106]. However, it was reported that some T. gondii strains partially or entirely lose their 529-bp repetitive units which can compromise the assay efficiency [107].

Nested-PCR utilizing the GRA7 gene has been proposed as a good target for primer design to detect T. gondii. A nested PCR assay with potential to detect clonal and atypical strains of T. gondii was developed by analyzing the genome of different strains of the parasite; this resulted in GRA7 being identified as the most appropriate target for amplification [100]. Many conserved regions are present throughout the GRA7 sequence which can be used for primer design. Moreover, GRA7 plays a significant role in the parasitophorous vacuole, involved in sequestration of host endolysosomes, and expressed in all infectious stages including tachyzoite, bradyzoite, merozoite, and sporozoite [108]. A nested PCR assay amplifying conserved regions in GRA7 demonstrated sensitivity and specificity comparable to the 529-bp fragment, and was more sensitive than the B1 gene; thus, GRA7 could prove to be an alternative target for amplification particularly when the genetic diversity of the parasite is of concern [100].

An isothermal amplification technique allows DNA amplification to be performed rapidly. Two of the techniques that have been used for the rapid detection of Toxoplasma are “Loop-Mediated Isothermal Amplification” (LAMP) and “Nucleic Acid Sequence-Based Amplification” (NASBA).

**Loop-mediated isothermal amplification**

LAMP provides an opportunity to amplify DNA rapidly with high specificity and efficiency under isothermal conditions (64 °C), thus serving as an RDT [109]. This method makes use of four specific primers that bind at six different sequences on the same target DNA. Unlike conventional PCR, this method is simple and inexpensive and does not need purified DNA for efficient amplification. The reaction can be accomplished in a single tube in a simple incubator, and the results can be read within one hour. The characteristic features like rapid amplification, simple operation, and easy read-out system are useful for robust and reliable detection of infection at the early stage [110]. This technique is a promising molecular detection approach to be used in developing countries where Toxoplasma infection is endemic. In humans, the LAMP method has been developed using three T. gondii genes, i.e., SAG1, SAG2, and B1 [111]. The sensitivity and specificity of the LAMP method were evaluated by comparing with conventional nested PCR; the former was found to be ten times more sensitive than the latter. The LAMP method showed a detection limit of 0.1 tachyzoite and no cross-reactivity with blood samples obtained from healthy individuals and patients with other parasitic infections such as Plasmodium spp. and Brugia malayi [111].

Species-specific LAMP primers have been designed using a T. gondii 529-bp sequence to improve the LAMP assay [110]. Compared to conventional PCR which detected 2.5% (5/200) of samples, the LAMP assay detected 7% (14/200) of samples. This assay has been produced as a commercial kit by DiaSorin®, called the IAM TOXO Q-LAMP kit, which was named later as IAm TOXO [112]. The IAm Toxo was reported to be highly sensitive and robust and gave good results compared to the alternative amplification technologies [112]. The LAMP method has also been used to detect primary Toxoplasma infection in high-risk pregnant women with previous spontaneous abortion history [113]. The clinical sensitivity and specificity of LAMP were compared with quantitative real-time PCR. Both techniques demonstrated 100% sensitivity and specificity using an advanced melting curve analysis. Furthermore, no positive results were obtained in samples that were extracted from other parasites including Giardia, Cryptosporidium, Blastocystis, Leishmania.
spp., and Plasmodium spp. [113]. Both techniques were then employed to detect recent *T. gondii* infection in 77/139 IgG negative women who suffered from spontaneous abortion. The result showed that both techniques were positive in eight samples, thus confirming primary *Toxoplasma* infection [113].

*Toxoplasma* infection has also been reported in malignancies such as lymphoma, leukemia, or multiple myeloma [114]. To devise a specific and accurate method for detecting *Toxoplasma* infection in the blood of children with leukemia, the technical performance of LAMP was compared with that of nested PCR by targeting two repetitive conserved regions (RE and B1) in the *T. gondii* genome [115]. The results showed that the LAMP assay was superior in terms of sensitivity and specificity using the same genomic DNA extracted from mice tachyzoites and blood samples of leukemic patients.

**Nucleic acid sequence-based amplification**

Nucleic acid sequence-based amplification (NASBA) is an isothermal method that has been proposed as an efficient diagnostic approach for amplifying RNA targets of viable cells and adapted in clinical diagnostics [116]. The NASBA method to detect live tachyzoites of *T. gondii* in the peritoneal cavities of mice based on the amplification of the tachyzoites’ B1 rRNA gene has been developed [117]. The amplified gene yielded an amplicon of 116 bp on an agarose gel. The application of real-time NASBA is advantageous since it accelerates the analysis, minimizes the steps and risk of contamination, and precludes errors during the process [118]. This method employs molecular beacons which are small, single-stranded hairpin nucleic acid probes. The beacons generate a fluorescent signal after binding to the target, and the amplicons can be directly detected in a single tube. The signal is then recorded by a fluorimeter, thereby eliminating the need for running an agarose gel. Moreover, the detection limit of NASBA was one parasite/milliliter of blood while that of RT-PCR was ten parasites/milliliter [118].

**Discussion**

Accurate detection of parasitic infection underpins a holistic approach to its control and management. Conventional ELISA is a reliable technique; however, it is time-consuming and cumbersome due to liquid handling, and needs expensive equipment and reagents along with trained technicians [119]. Inexpensive, easy-to-use, rapid, sensitive, and specific detection tools may help to reduce the burden and impact of diseases in low-resource areas. Despite the high demand, a relatively limited number of validated rapid diagnostics are commercially available for parasitic infections, including *Toxoplasma*. The LFT strip, as the most commonly available POC test, has several advantages, including its rapidity in getting results, cost-effectiveness, application at point-of-care, and long shelf life; does not need refrigeration especially during transportation; and pretreatment is usually not required for fluid samples [120]. Some of the shortcomings of the LFT strips include the fact that majority are qualitative tests, they are not high throughput, most are not multiplexed, and there may be obstruction of the pores due to the matrix components [120, 121].

*T. gondii* recombinant proteins have demonstrated significant specificity and sensitivity in detection assays. However, none of the assays based on the recombinant antigens seemed to be able to replace native tachyzoite antigen in IgG/IgM-based tests. For instance, the diagnostic sensitivities for IgM detection of two commercial POC tests which use recombinant *T. gondii* antigen, i.e., Biopanda Toxo IgG/IgM and OnSite Toxo IgG/IgM, were found to be much lower compared to that of the LDBIO Toxoplasma ICT IgG-IgM test which makes use of whole-cell lysate of tachyzoites from the *T. gondii* RH Sabin Type I strain [35].

Chimeric antigens are composed of different distinct immunoreactive epitopes of *T. gondii* antigens, and it is likely that serum antibodies from a *Toxoplasma*-infected person will bind with at least one of the epitopes [122, 123]. Therefore, the application of a chimeric recombinant antigen is considered potentially more efficient than that of single antigens for developing a diagnostic method for *Toxoplasma* infection [122, 124]. Two “artificial” antigens named as EC2 and EC3 containing six distinct regions of MIC2, MIC3, SAG1, GRA3, GRA7, and M2AP have been genetically engineered. All the individual polypeptides in both chimeric antigens retained their antigenic properties in IgG/IgM ELISA [125]. Both improved the serological diagnosis of *Toxoplasma* infection in adults with acquired infection and infants with primary infection, indicating that the gene fusion did not affect the antigenic properties of each epitope in the chimeric format. Compared to the commercial assays [ELFA-IgM (bioMérieux, France) or ETI-TOXOK-M (DiaSorin, Italy)] which detected only 35% of the infected infants, IgM-ELISA utilizing GST-EC2 and GST-EC3 detected 70% of serum samples of infants with congenital *Toxoplasma* infection [125]. In another example, a chimeric antigen comprising immunodominant regions of MIC1, MAG1, and SAG1 demonstrated better results for the detection of serum IgG than by ELISA using *Toxoplasma* lysate antigen [126]. Thus, the chimeric antigen can substitute the preparation of parasite lysate in clinical diagnostic tests [127]. To date, a chimeric antigen has not been used in developing an RDT; however, it can be envisaged that chimeric antigens which perform well in IgG/IgM-ELISA can be readily developed into RDTs, including those capable of differentiating between recently acquired and past infection. Based on the market need, biomarkers for *Toxoplasma* infection can also be combined with biomarkers of other relevant infections in a multiplex POC immune chip.
The unique electrical, magnetic, luminescent, and catalytic properties of nanomaterials allow rapid, sensitive, and inexpensive diagnosis of microbial infections, and confer the potential for real-time detection of target biomarkers in a small volume of patient sample [128]. Also, advances in nanotechnology, microfluidics, biosensors, and synthetic biology have given rise to the generation of miniature-sized laboratory systems known as “lab-on-chip” devices [129]. It can also address the demand of nucleic acid testing at the POC level that integrates all the steps from sample preparation to nucleic acid amplification and detection in a single device [130]. The advantages of multifunctional microfluidic lab-on-chip technology include robustness, small sample volume, enhanced reproducibility, fast analysis, accurate quantification and automation of all steps from sample preparation to signal generation and detection. Currently, the microfluidic chip is still expensive due to manufacturing procedures, non-scalability, and requirement of a microscopic syringe pump for operation. It is thus not surprising that such a device is still not available commercially for Toxoplasma detection. Researchers are still striving to produce POC lab-on-chip devices that are more affordable and suitable for low resource settings and mass screening [131]. We can thus look forward to the future availability of highly sensitive, specific, portable, and inexpensive, rapid diagnostic devices for Toxoplasma infection in humans and animals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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