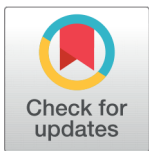


Diagnostic methods and protocols used in investigating *Toxoplasma gondii* in humans: A review

Shameeran S. Ismael

Department of Medical Laboratories Sciences, College of Health Sciences, University of Duhok, Duhok, Iraq



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Corresponding Author

Shameeran S. Ismael
shameeran.ismael@uod.ac

Department of Medical
Laboratories Sciences, College
of Health Sciences, University of
Duhok, Duhok, Iraq

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ABSTRACT

Toxoplasmosis, a zoonotic disease, is a parasitic infection caused by a coccidian protozoan *Toxoplasma gondii*. In immunocompetent people, the infection is asymptomatic, while it can produce serious signs in immunocompromised people and in developing fetuses. Accurate diagnosis is depended, mainly, on the clinical features. However, in immunocompromised patients, the diagnosis is very difficult and may lead to misdiagnosis and improper treatment. Today, the molecular diagnosis and serotyping are widely used for the diagnosis of *T. gondii* in many countries. The aim of the present short review is to highlight the current diagnostic methods and protocols that are used for the diagnosis of *T. gondii* in humans.

Keywords molecular diagnosis, serological diagnosis, serotyping, *T. gondii*, toxoplasmosis

INTRODUCTION

Toxoplasma gondii is an obligate intracellular, zoonotic protozoan parasite that infects humans and other warm-blooded species and causes a serious disease called toxoplasmosis. Intermediate hosts for the *T. gondii* are humans and other warm-blooded species whereas cats and other felines are the definitive hosts.^{1,2} This parasite is widely spread through the human population and affecting more than a billion people, globally.³ As a parasite, *T. gondii* invades the host's nucleated cells to get nutrients and for replication. The common route of transmission of toxoplasmosis is by ingestion of undercooked red meat containing bradyzoite (tissue cysts), by ingestion of contaminated food, grasses or drinking of contaminated water with the infective stage sporulated oocyst. Toxoplasmosis is considered a disease of pregnancy by multiplying in the placenta and fetus and causing an acute and potentially fatal disease.⁴⁻⁶ Although toxoplasmosis is usually benign, the congenital disease can lead to severe complications for the fetus and baby, such as the death of the fetus in the uterus, miscarriage in the early stages of pregnancy or cause some neurological lesions including hydrocephalus, microcephaly, where the infection occurs in the late stage of pregnancy.⁷

Generally, there are many factors that affecting the prevalence and incidence of toxoplasmosis including nutritional status, age, culture and hygiene habits.⁷ Toxoplasmosis in adults and children is asymptomatic in most cases, and lymphadenopathy is the most common symptom in the 10%–20% of other-wise immunocompetent persons who have a primary *T. gondii* infection that is symptomatic. Chorioretinitis, myocarditis, and polymyositis are some of the less prevalent manifestations in these patients.^{8,9} Antibodies to *T. gondii* can be found in serum samples from any host, indicating that the host has been infected previously. Toxoplasmosis symptoms that aren't clinically or typically specific aren't enough to make a definitive diagnosis.¹⁰ In this context, accurate diagnosis and treatment at the proper time are still the main ways to get toxoplasmosis off.

There are many methods and techniques used for the diagnosis of toxoplasmosis in humans. This review aims to highlight the currently used methods for the diagnosis of *T. gondii* infection.

CURRENT METHODS FOR THE DETECTION OF *T. GONDII*

Detection of the acute phase of infection can be determined by serological methods. The IgG response normally appears 7 to 14 days after the infection and reaches peak in 30 to 60 days, and generally persists for a long time. After primary infection, IgM appears before IgG but normally does not remain; if present, it indicates acute infection; nonetheless, confirmatory tests should be performed because its specificity is not always sufficient.¹¹ The initial and major way for the diagnosis is the use of serologic assays to demonstrate the presence of specific antibodies to *T. gondii*. Various serologic tests frequently measure different antibodies, each of which has a distinct rise and fall pattern with time after infection. To determine whether an individual has been infected in the distant past or has been infected recently, a combination of serologic tests is usually required. These difficulties must be familiar to physicians and clinical laboratory scientists, and if necessary, reference laboratories must be consulted.¹²

There are several serological tests that are used for the diagnosis and detection of humoral antibodies including latex agglutination test, indirect agglutination test, indirect fluorescent assay (IFA), immunosorbent agglutination assay, direct agglutination test, Sabin–Feldman dye test and an enzyme-linked immunosorbent assay (ELISA).

The first serological test is the latex agglutination test (LAT), which is widely used and considered as a screening test for the detection of *T. gondii* antibodies. LAT has advantages and disadvantages, mainly, it is simple, sensitive, specific and inexpensive. The disadvantages of this test imply that it be used with caution in screening immunocompromised individuals and pregnant women living in regions where the infection is uncommon due to its moderate positive predictive value.¹³

The second serological test is the Sabin–Feldman dye test or dye test (DT) which was the first test used for the detection of all antibodies against toxoplasmosis infection and till

now is considered as a gold standard.^{14,15} The main advantage of dye test in human is both specific and sensitive, but it may be unreliable in animals. The most significant disadvantage of dye test is that it requires live parasites and healthy human serum as an accessory factor, greatly limiting its availability.¹⁶

The western blot technique has been used to distinguish between maternal-specific antibodies and those generated by the newborn infant within the first six months of life, in addition to classical laboratory techniques.^{17,18}

The third serological test is enzyme-linked immunosorbent assay (ELISA), which is used for the detection of *T. gondii* antibodies. ELISA test is not always typical because the production of the antibodies may fail to produce or takes a lot of time. Major cases of active toxoplasmosis are occurred due to the reactivation of latent infections.¹⁹

The last serological method used for the diagnosis of *T. gondii*, is the serotyping of *T. gondii*, which is quick and inexpensive and does not need the isolation of *T. gondii* from the sample. There are several methods for typing of *T. gondii* in humans and animals, including ELISA, recombinant antigens and peptide-microarray tests.^{20,21}

While the diagnosis of toxoplasmosis is not always dependable on clinical signs and serological result, the molecular diagnosis is done, when the serological diagnosis is not conclusive. Real-time polymerase chain reaction (RT-PCR) is a sensitive and promising method capable of yielding a quantitative result.^{22,23} For the detection of the *T. gondii* in biological samples, there are three repetitive DNA sequences depend on the repeat length and array size and classified as the following; the 35-copy *BI* gene, the 300-copy 529 bp repeat element and the 110-copy internal transcribed spacer (ITS-1).²⁴

For the diagnosis of toxoplasmosis, several PCR techniques have been developed, that are using a variety of clinical specimens including amniotic fluid, blood, cerebrospinal fluid, etc.²⁵ Conventional PCR and RT-PCR are widely used to identify the genotypes of strains of *T. gondii*, because there are three human types including: types I, II and III. Genotyping and characterization of strains of *T. gondii* relay on sequencing and phylogenetic tree analysis.²⁶ PCR amplification for the detection of *T. gondii* DNA in body fluids and tissues has been successfully utilized to diagnose congenital, ocular, and cerebral toxoplasmosis. Early detection of *T. gondii* DNA in intrauterine by PCR technique is very important to avoid the necessity of more intrusive techniques on the fetus.

Finally, histological examination of the stained prepared smears from cerebrospinal fluid (CSF) or from amniotic fluid with Giemsa or Wright stains for the demonstration of trophozoites (tachyzoites) in the acute stage of infection, is another method for diagnosis of toxoplasmosis, the demonstration of trophozoites in tissue section after staining is very difficult compared to body fluid prepared stained smears.²⁷

There are some other techniques and methods that can also be used to assess the infection such as the histology of infected tissues, PCR of body fluids or culturing of the *T. gondii*.

CONCLUSIONS

This review aimed to give a short presentation of current and emerging serological and molecular methods are used for the detection and diagnosis of toxoplasmosis and typing of isolated *T. gondii*. The accurate and early diagnosis of toxoplasmosis is critical for proper therapy. The microscopic detection of *T. gondii* is considered the gold standard. Serological assays for the detection of *T. gondii*-specific antibodies or circulating antigens have been established, and several serological tests have been established for the detection of *T. gondii*-specific antibodies or circulating antigens. In addition to serological methods, molecular techniques, mainly PCR, depend on the DNA or RNA amplifications are widely used for the identification of *T. gondii*. Finally, the potential way for typing of *T. gondii* in humans and animals is by serotyping methods and these depend on the polymorphic polypeptides.

DECLARATIONS

Conflicts of interest

The author has no conflict of interest.

Ethical approvals

Not required for the review articles.

Data availability

Not applicable.

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