Laboratory Diagnosis of *Toxoplasma gondii* Infection and Toxoplasmosis

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For the past 40 years, the *Toxoplasma* Serology Laboratory at the Palo Alto Medical Foundation Research Institute (TSL-PAMFRI) has been dedicated to the laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. TSL-PAMFRI is the “brain child” of Jack S. Remington. Jack’s ceaseless devotion to objectivity and uncompromising excellence has made TSL-PAMFRI the *Toxoplasma* reference laboratory for the Centers for Disease Control and Prevention, the US Food and Drug Administration, and health care providers and clinical laboratories in the United States and other countries. Jack’s leadership and vision created, defined, and significantly contributed to the development of laboratory methods for the diagnosis of the infection and diseases caused by *T. gondii*. A summary of the laboratory tests currently available at TSL-PAMFRI for the diagnosis of infection and disease caused by the parasite is presented here.

**General Considerations**

The term toxoplasmosis is reserved to describe the clinical or pathological disease caused by *Toxoplasma gondii* and *T. gondii* infection for an asymptomatic primary infection or persistence of the parasite in tissues (chronic or latent infection) [1].

When considering toxoplasmosis in the differential diagnosis of a patient’s illness, it is important to keep in mind that emphasis should not be placed on whether the patient has or has not been exposed to cats. Transmission of parasites essentially occurs without knowledge of the patient and may be unrelated to direct exposure to a cat (e.g., by ingestion of vegetables or water contaminated with oocysts or ingestion of undercooked meat contaminated with cysts). On the other hand, patients with an indoor cat that is fed only cooked food is not at risk of acquiring infection from that cat. Serologic investigation of a cat to establish whether it is a potential source of the infection should be considered. The prevalence of *T. gondii* antibodies among cats in a given locale is usually similar to their prevalence in humans. Seropositivity in the cat does not predict shedding of oocysts.

For clinical purposes, toxoplasmosis can be divided into five infection categories, including those (1) acquired by immunocompetent patients, (2) acquired during pregnancy, (3) acquired congenitally, and (4) acquired by or reactivated in immunodeficient patients, and including (5) ocular infections. In any category, clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered. Furthermore, methods of diagnosis and their interpretations may differ for each clinical category.

**Diagnostic Methods**

The diagnosis of *T. gondii* infection or toxoplasmosis may be established by serologic tests, amplification of specific nucleic acid sequences (e.g., polymerase chain reaction [PCR]), histologic demonstration of the parasite and/or its antigens (e.g., immunoperoxidase stain), or by isolation of the organism [1]. Other rarely used methods include demonstration of antigenemia and antigen in serum and body fluids, a toxoplasmin skin test, and antigen-specific lymphocyte transformation [1].

**Serologic Tests**

The use of serologic tests for demonstration of specific antibody to *T. gondii* is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection [2]. A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the recent or more distant past or has been recently infected. The clinician and clinical laboratories must be familiar with these problems and consult reference laboratories if the need arises.

A panel of tests (the *Toxoplasma* Serological Profile [TSP]) consisting of the Sabin-Feldman dye test (DT) [3], double sandwich IgM ELISA [4], IgA ELISA [5], IgE ELISA [6, 7], and AC/HS test [8] has been used successfully by our group to determine if serologic test results are more likely consistent with infection acquired in the recent or more distant past [2, 9, 10]. The AC/HS test is interpreted as previously described [8] by comparing IgG titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen).

The TSP has been successfully used in the setting of toxoplastic lymphadenitis [2], myocarditis [11], polymyositis [11], and chorioretinitis [10] and during pregnancy [9]. For sera with positive results in IgG and IgM tests, the discriminatory power...
of the TSP to differentiate between recently acquired infection and chronic infection is probably superior to any other single serologic test.

Current interpretation of results in the TSP at the Toxoplasma Serology Laboratory at the Palo Alto Medical Foundation Research Institute (TSL-PAMFR1) is as follows: Sera that are positive in the DT, negative in the IgM, IgA, and IgE ELISAs, and reveal a chronic pattern in the AC/HS test are typically found in patients infected in the most distant past. The combination of high titers in the DT, positive IgM, IgA, and IgE ELISAs, and an acute pattern in the AC/HS test is highly suggestive of a recently acquired infection. In contrast, the presence of positive DT and IgM ELISA results but a negative, low-positive, or equivocal result in the IgA and IgE ELISAs and an equivocal pattern in the AC/HS test is more difficult to interpret. In the latter setting, a follow-up sample is usually obtained, the 2 samples are run in parallel, and the serologic test titer results are compared. If the titers obtained in the 2 samples do not change significantly, the infection is most likely to have been acquired in the distant past. In contrast, significant changes (rise or decline) detected in the titers of the 2 samples are considered to be suggestive of a recently acquired infection.

**IgG antibodies.** The most commonly used tests for the measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test [1, 12]. In these tests, IgG antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life.

When two different compounds (i.e., acetone and formalin) are used to fix parasites for use in the agglutination test, a “differential” agglutination test (also known as the “AC/HS test”) results due to the fact that the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection. This test has proved useful in helping to differentiate acute from chronic infections [8] but is best used in combination with a panel of other tests (e.g., the TSP).

Recently, a number of tests for avidity of Toxoplasma IgG antibodies have been introduced to help discriminate between recently acquired and distant infection [13–15]. It has been observed that the functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and that it increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated samples.

**IgM antibodies.** IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. The most commonly used tests for the measurement of IgM antibody are double-sandwich or capture IgM-ELISA kits [4], the IFA test, and the immunsorbent agglutination assay (IgM-ISAGA; available from bioMérieux) [1]. False-positive results due to rheumatoid factor and antinuclear antibodies in some IgM-IFA tests are not detected by the most commonly used commercial double-sandwich or capture IgM-ELISAs [4]. Despite the wide distribution of commercial test kits to measure IgM antibodies, these tests often have low specificity, and the reported results are frequently misinterpreted [16, 17].

An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past, and because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed.

In patients with recently acquired primary infection, T. gondii–specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some patients, positive T. gondii–specific IgM titers can still be observed during the chronic phase of infection [16]. Some investigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection [18]. The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected. Further complicating the interpretation of a positive IgM test result is the fact that several methods for its detection still may result in a relatively high frequency of false-positive results [16, 17]. Thus, a positive IgM test result in a single serum sample can be interpreted as a true-positive result in the setting of a recently acquired infection, a true-positive result in the setting of an infection acquired in the distant past, or a false-positive result.

**IgA antibodies.** IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants by use of ELISA or ISAGA [5]. As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than a year. For this reason, they are of little additional assistance for diagnosis of acute infection in the adult. In contrast, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis of the infection in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serologic diagnosis has been established by the presence of IgA and IgG antibodies [5].

**IgE antibodies.** IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmonic chorioretinitis [6, 7]. Their demonstration does not appear to be particularly useful for diagnosis of T. gondii infection in the fetus or newborn when compared with IgA tests. The duration of IgE seropositivity is briefer than that with IgM or IgA antibodies and hence appears useful for identifying recently acquired infections [2, 7].

**PCR**

PCR amplification for detection of T. gondii DNA in body fluids and tissues has successfully been used to diagnose congenital [19], ocular [20, 21], and cerebral and disseminated [22, 23] toxoplasmosis. PCR has revolutionized the diagnosis of intrauterine T. gondii infection by enabling an early diagno-
sis to be made, thereby avoiding the use of more invasive procedures on the fetus. PCR has enabled detection of *T. gondii* DNA in brain tissue [24], cerebrospinal fluid (CSF) [25], vitreous and aqueous fluids [20], bronchoalveolar lavage (BAL) fluid [26], and blood [23] in patients with AIDS.

**Histologic Diagnosis**

Demonstration of tachyzoites in tissue sections or smears of body fluid (e.g., CSF or amniotic or BAL fluids) establishes the diagnosis of the acute infection [1]. It is often difficult to demonstrate tachyzoites in conventionally stained tissue sections. The immunoperoxidase technique, which uses antisera to *T. gondii*, has proven both sensitive and specific. It has been used successfully to demonstrate the presence of the parasite in the central nervous system (CNS) of AIDS patients [27]. The immunoperoxidase method is applicable to unfixed or formalin-fixed paraffin-embedded tissue sections [27]. A rapid, technically simple, and under-used method is the detection of *T. gondii* in air-dried, Wright-Giemsa–stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue. Multiple tissue cysts near an inflammatory necrotic lesion probably establish the diagnosis of acute infection or reactivation of latent infection.

**Isolation of *T. gondii***

Isolation of *T. gondii* from blood or body fluids establishes that the infection is acute. Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid.

**Diagnosis of Specific Clinical Entities**

The first step in pursuing the diagnosis of *T. gondii* infection or toxoplasmosis is to determine whether the individual has been exposed to the parasite. In essentially all cases, any of the tests for the detection of IgG antibodies reliably establish the presence or absence of the infection. In a small number of patients, IgG antibodies might not be detected within 2–3 weeks after the initial exposure to the parasite; however, this is rare. In addition, rare cases of toxoplasmic chorioretinitis and toxoplasmic encephalitis in immunocompromised patients have been documented in patients with negative *T. gondii*–specific IgG antibodies.

The second step consists of establishing whether the patient has a recently acquired infection or an infection acquired in the more distant past. In general, a true-negative IgM test essentially rules out that the infection has been acquired in recent months. A positive IgM test is more difficult to correctly interpret. One must not assume that a positive IgM test result is diagnostic of recently acquired infection (see above under *IgM antibodies* in the Serologic Tests section). Confirmatory testing should be done for all cases for whom IgM test results are positive [9, 17, 28]. Serologic tests should not be considered useful for measuring response to therapy.

The third step is to establish whether the patient’s condition or illness is due to toxoplasmosis (recently acquired infection or recrudescence of latent infection) or is unrelated to the infection.

**Toxoplasmosis in the Immunocompetent Patient**

The vast majority of cases of *T. gondii* infection in adults and children are asymptomatic [29]. Lymphadenopathy is the most common manifestation in the 10%–20% percent of otherwise immunocompetent individuals whose primary *T. gondii* infection is asymptomatic. Less common presentations in these patients include, but are not limited to, chorioretinitis, myocarditis, and/or polymyositis [30].

Tests for IgG and IgM antibodies should be used for initial evaluation of these patients. Testing of serial specimens obtained 3–4 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal. Negative results in both tests virtually rule out the diagnosis of toxoplasmosis. In rare instances early in infection, IgG antibodies may not be detectable, whereas IgM antibodies are present (hence the need for both tests to be performed). Acute infection is supported by documented seroconversion of IgG and IgM antibodies or a greater than four-fold rise in IgG antibody titer in sera run in parallel. A single high titer of any immunoglobulin is insufficient to make the diagnosis since IgG antibodies may persist at high titers for many years [1] and IgM antibodies may be detectable for >12 months. The TSP, performed on a single serum sample, is useful in determining the likelihood that the infection is acute.

Characteristic histologic criteria and a TSP consistent with recently acquired infection establish the diagnosis of toxoplasmic lymphadenitis in older children and adults [2, 31].

Endomyocardial biopsy and biopsy of skeletal muscle has been successfully used to establish *T. gondii* as the etiologic agent of myocarditis and polymyositis in immunocompetent patients [11]. Isolation studies and PCR have rarely proven useful for diagnosis in immunocompetent patients.

**Ocular Toxoplasmosis**

Toxoplasmic chorioretinitis may result from congenital or postnatally acquired infection. In both of these situations, lesions may occur during the acute or latent (chronic) stage of the infection [10, 32, 33].

Low titers of IgG antibody are usual in patients with active chorioretinitis due to reactivation of congenital *T. gondii* infection; IgM antibodies usually are not detected. When sera from such patients are examined by use of the DT, titers should be first determined with undiluted serum since in some cases, the conventional initial dilution of 1:16 may be negative.
In most cases, toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empiric therapy directed against the organism is often instituted on the basis of clinical findings and serologic test results. In a number of patients, the morphology of the retinal lesion(s) may be non-diagnostic and/or the response to treatment is suboptimal. In such cases (unclear clinical diagnosis and/or inadequate clinical response), detection of a local and increased T. gondii antibody response in ocular fluids (immune load), demonstration of the parasite by isolation or histopathology, or amplification of T. gondii DNA (in both aqueous and vitreous fluids) have been used successfully to establish the diagnosis [21, 34, 35].

Toxoplasmosis in the Immunodeficient Patient

In contrast to the relatively favorable course of toxoplasmosis in almost all immunocompetent individuals, immunologically impaired patients usually develop a dreadful and often life-threatening disease [36]. Immunocompromised patients at higher risk for toxoplasmosis include those with hematologic malignancies (particularly patients with lymphoma), bone marrow transplant, solid organ transplant (including heart, lung, liver, or kidney), or AIDS.

Toxoplasmic encephalitis is the most common presentation of toxoplasmosis in immunocompromised patients [36] and is the most frequent cause of focal CNS lesions in AIDS patients [37]. It is unclear whether T. gondii penetrates the brain more easily than other organs or whether it is more difficult for the brain, as an immunologically privileged site, to eradicate the organism during the initial acute infection and once residual infection has been established [38]. A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are observed in patients with toxoplasmic encephalitis [39]. Other organs commonly involved in immunocompromised patients with toxoplasmosis are the lungs, eyes, and heart.

In the vast majority of immunocompromised patients, toxoplasmosis results from reactivation of a latent infection. In contrast, in heart transplant patients and in a small number of other immunocompromised patients, the highest risk of developing disease is in the setting of primary infection (i.e., a seronegative recipient who acquires the parasite from a seropositive donor via a graft) [40, 41].

Because reactivation of chronic infection is the most common cause of toxoplasmosis in patients with malignancies or AIDS or in recipients of organ transplants (other than heart transplants), initial assessment of these patients should routinely include an assay for T. gondii IgG antibodies. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected.

When toxoplasmosis is suspected in immunocompromised patients chronically infected with the parasite (those with documented positive T. gondii–specific IgG antibody prior to the onset of immunosuppression) additional serologic testing adds very little (or may be misleading) to the diagnostic evaluation [41, 42]. In these patients, results indicating apparent reactivation (rising IgG and IgM titers) may be present in the absence of clinically apparent infection. In addition, serologic test results consistent with chronic infection may be seen in the presence of toxoplasmosis [36, 42]. Thus, for immunocompromised patients in whom toxoplasmosis is suspected, additional diagnostic methods to attempt to establish the diagnosis are strongly recommended. These methods include PCR amplification for detection of T. gondii DNA in blood or body fluids suspected of being infected, isolation of the parasite from blood or body fluids that may contain the parasite, and histologic examination of available tissues with T. gondii–specific stains, such as immunoperoxidase.

When clinical signs suggest involvement of the CNS and/or spinal cord, tests should include computed tomography or magnetic resonance imaging (MRI) of the brain and/or spinal cord. Neuroimaging studies of the brain should be considered even if the neurologic examination does not reveal focal deficits. Empiric anti–T. gondii therapy for patients with multiple ring enhancing brain lesions (usually established by MRI), positive IgG antibody titers against T. gondii, and advanced immunodeficiency (i.e., CD4 cell count of <200 cells/mm 3 ) is accepted clinical practice; a clinical and radiologic response to specific anti–T. gondii therapy is considered as supportive of the diagnosis of CNS toxoplasmosis. Brain biopsy should be considered in immunocompromised patients with presumed CNS toxoplasmosis if there is a single lesion on MRI, a negative IgG antibody test result, or inadequate clinical response to an optimal treatment regimen or to what the physician considers to be an effective prophylactic regimen against T. gondii (e.g., trimethoprim-sulfamethoxazole) [30]. If T. gondii serologic and radiologic studies do not support a recommendation for empiric treatment or are inconclusive and if brain biopsy is not feasible, a lumbar puncture should be considered if it is safe to perform; PCR can then be performed on the CSF specimen. CSF can also be used for isolation studies, although it is uncommon for T. gondii to be isolated from CSF from immunocompromised patients. Of note, PCR examination of CSF can also be used for detection of Epstein-Barr virus, JC virus, or cytomegalovirus DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy, or cytomegalovirus ventriculitis, respectively, have been considered in the differential diagnosis.

T. gondii Infection in Pregnancy

T. gondii infection acquired during pregnancy may result in severe damage or death of the fetus and long-term sequelae in offspring. Because congenital toxoplasmosis results almost solely in women who acquire the infection during gestation, it is critical to determine whether infection during pregnancy has occurred. The incidence of congenital toxoplasmosis in the off-
spring of women infected prior to gestation has been shown to 
be extremely rare unless a woman is immunocompromised 
(e.g., receiving corticosteroids or immunosuppressive drugs 
or positive for human immunodeficiency virus [HIV]).

At present in the United States, definitive diagnosis of the 
acute infection and the time of its occurrence have been com-
promised by the lack of systematic screening and the fact that 
only a single serum sample is submitted for testing. When only 
1 serum sample is available, tests to detect the presence of IgG 
and IgM antibodies are most commonly used to determine if a 
pregnant woman acquired acute infection during gestation.

A negative IgM test result for a pregnant woman in the first 
24 weeks of gestation with a low IgG test titer (i.e., DT 
pregnant woman acquired acute infection during gestation. 
and IgM antibodies are most commonly used to determine if a 
only a single serum sample is submitted for testing. When only 
acute infection and the time of its occurrence have been com-
pling of pregnant women in their first trimester. In the third 
avidity antibodies are detected and the serum is obtained during 
ting is only a confirmatory test and not the ultimate test for 
decision making. Its highest value is observed when high IgG 
avidity antibodies are detected and the serum is obtained during 
the time window of exclusion of acute infection for a particular 
method (i.e., 12 weeks for the Labsystems method and 16 
weeks for the Vidas immunoassay [bioMérieux]).

Once the diagnosis of acute acquired infection during preg-
nancy has been presumptively established, diagnostic efforts 
should then focus on determining whether the fetus has been 
infected.

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis. Prenatal diagnosis of fetal infection is 
advised when a diagnosis of acute infection is established or 
highly suspected in a pregnant woman or an abnormality in 
the fetus suggests congenital toxoplasmosis. Methods to obtain 
fetal blood, such as periumbilical fetal blood sampling, have 
been largely abandoned because of the risk involved for the 
fetus and the delay in obtaining definitive results with conven-
tional parasitologic tests [43].

Prenatal diagnosis of congenital toxoplasmosis is currently 
based on ultrasonography and amniocentesis. PCR on amniotic 
fluid for detection of T. gondii–specific DNA performed from 
18 weeks onwards of gestation should be used in all cases of es-
established acute maternal infection or cases with serologic 
test results highly suggestive of acute acquired infection during 
pregnancy [43]. In a recent report, the overall sensitivity of PCR on 
amniotic fluid was estimated to be 64%, the negative predictive 
value was estimated to be 87.8%, and specificity and positive 
predictive value were estimated to be 100% [44]; in this study, 
marked differences in sensitivity were observed depending on 
the gestational age at the time of the amniocentesis [44]. The 
reliability of the PCR test before 18 weeks of gestational age is 
unknown [43, 44]. PCR on amniotic fluid is not recommended 
for HIV-infected women because of the risk of transmitting the 
HIV virus to the fetus during the amniocentesis procedure.

Diagnosis in the newborn. Maternal IgG antibodies present 
in the newborn may reflect either past or recent infection in the 
mother. For this reason, tests for detection of IgA and IgM anti-
bodies are commonly employed for diagnosis of infection in the 
newborn. Serum samples obtained from umbilical cord should 
be used as they may be contaminated with maternal blood. Demonstration 
of IgA antibodies appears to be more sensitive than detection of 
IgM antibodies for establishing infection in the newborn [5]. T. 
gondii–specific IgM may be present when there is no T. gondii– 
specific IgM, and the converse may also occur. If IgA antibodies
Toxoplasmonic Encephalitis

Introduction

Toxoplasma gondii, an ubiquitous intracellular parasite, can severely damage the brain of humans; it is one of the most common infectious agents of humans throughout the world. It is transmitted primarily via the oral route and the definitive hosts are members of the cat family (1). The primary encounter between this intracellular pathogen and its incidental human host most often goes unrecognized. In approximately 10% of immunocompetent adults and children it causes a self-limited and non-specific illness that rarely requires treatment. The most frequently observed clinical manifestation is lymphadenopathy and fatigue without fever (1, 2). Immunity in the immunocompetent host is lifelong. Reinfection does not seem to result in clinically apparent disease. In almost all cases, this primary event leads to a chronic or latent infection which may remain in a state of infection without clinical significance.

In contrast, in severely immunocompromised individuals, the reactivation of the chronic infection causes toxoplasmosis, a disease that can affect almost any organ in the body. The central nervous system (CNS) is the most seriously affected tissue by T. gondii.
lymphadenopathy (MP). After 12 months all IgM ELISA titers were negative.

The IgA was positive during the first 6 months in 63% of the samples (Figure 3). Mean IgA ELISA titers (±SEM) decreased from 7.0 (±1.6) at 3 months to 3.8 (± 0.9) at 4 months (p value > 0.05) and rebound from this titer at 4 months to 5.8 (± 1.7) at 5 months (p value > 0.05). The titers declined thereafter as it was observed with the IgM titers (Table 2). Between 4 and 12 months, in 18 patients with negative IgM ELISA titers, 8 patients had a positive IgA ELISA test. A positive IgA ELISA titer of 3.4 was seen as late as 8 months after clinical onset of lymphadenopathy (MP). After 12 months all IgA ELISA titers were negative.

The IgE ELISA test was positive during the first 2 months in ≥ 85% of the sera studied (Figure 4). Between 4 and 12 months, in 18 patients with negative IgM ELISA titers, 5 patients had a positive IgE ELISA test. A positive IgE ELISA titer of 5 was seen as late as 8 months after clinical onset of lymphadenopathy (MP). After 12 months all IgE ELISA titers were negative.

The IgE ISAGA test was positive during the first 2 months in ≥ 80% of the samples (Figure 5). Mean IgE ISAGA titers (±SEM) fell from 8.3 (± 1.2) at 3 months to 3.5 (± 1.2) at 4 months (p value < 0.05) and rebound from this titer at 4 months to 7.8 (± 2.1) at 5 months (p value > 0.05). The titers declined thereafter as it was observed with the IgM and IgA ELISA titers (Table 2). Between 4 and 12 months, in 18
IgM antibodies. This was especially true for IgE antibodies. This early decline in IgE antibodies has been demonstrated by others (31-33). In patients with toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis T. gondii-specific IgG levels tend to be high, the DT was positive in each of the 22 patients and was at a high titer (≥ 1:1024) in 21 (95.5%) of them (7). In contrast, late manifestations of toxoplasmic chorioretinitis as a result of congenital infection are associated with T. gondii antibody titers that are not higher than those in individuals in the population without eye disease (2, 10, 26-30).

Isolation of the parasite from ocular tissues and fluids have been rarely attempted (12, 34). The yield of attempting to isolate T. gondii from ocular tissues is so low that it does not justify the invasive nature of a procedure like retinal biopsy or anterior chamber paracentesis to obtain a specimen for such a purpose. Of note, Remington and Little (34) attempted to isolate T. gondii at autopsy from eye specimens from 105 cases. In all of the 210 eyes examined, the isolation attempt was negative.
are detected in the newborn, the test should be repeated at ~10 days after birth to make certain that what is being measured is not contaminating maternal IgA antibodies. In addition, if the newborn has received a blood transfusion, serologic tests may measure exogenously administered rather than endogenous antibody.

Infants born to mothers chronically infected with *T. gondii* will have maternal *Toxoplasma*-specific IgG antibodies detected in their peripheral blood. In these infants, *Toxoplasma* serologic tests for IgM and IgA antibodies are usually negative, and efforts to detect *T. gondii* in body fluids or tissues (by isolation or PCR) should yield negative results. Follow-up serologic testing should be done on these patients until the IgG antibodies become undetectable. Maternally transferred IgG antibodies should disappear within the first 6–12 months of life. A negative *T. gondii*-specific IgG test result at 1 year of age essentially rules out congenital toxoplasmosis.

Additional diagnostic methods that have been used successfully to diagnose the infection in infants include direct demonstration of the organism by isolation of the parasite (e.g., mouse inoculation or inoculation in tissue cultures of CSF, urine, placental tissue, or peripheral blood) and amplification of *T. gondii*-specific DNA (e.g., PCR in CSF, peripheral blood, or urine) [1]. Evaluation of infants with suspected congenital toxoplasmosis should always include ophthalmologic examination, non-contrast computed tomography or ultrasound of the brain (to determine whether hydrocephalus or calcifications are present), and examination of CSF [1]. Detection of calcifications in the brain of a newborn by x-ray, ultrasound, or computed tomography should heighten the suspicion of *T. gondii* as the cause of the disease. In severely affected infants with congenital toxoplasmosis, unilateral or, more often, bilateral and symmetric dilatation of the ventricles is not an uncommon finding [1]. Persistent CSF pleocytosis and elevated protein content should lead the physician to consider a diagnosis of congenital toxoplasmosis even in subclinical cases [1].

Although not clinically available, antigen-specific lymphocyte transformation and lymphocyte typing in response to exposure to *T. gondii* antigens has been used successfully to diagnose the congenital infection in infants ≥2 months of age [45, 46]. Specific lymphocyte anergy to the organism may also occur in congenitally infected infants [47].

**Summary**

The diagnosis of *T. gondii* infection or toxoplasmosis can be established by serologic tests, PCR, histologic examination, or isolation of the parasite. *T. gondii* infection can be asymptomatic, and the clinical manifestations of patients with symptomatic toxoplasmosis are protean and nonspecific. The choice of the appropriate diagnostic method(s) and its (their) interpretation may differ for each clinical category (i.e., immunocompetent vs. immunodeficient patient). Reference laboratories should be contacted prior to diagnostic procedures to optimize the choice and handling of the specimens and their yield.

At the dawn of the twenty-first century, TSL-PAMFRI offers state-of-the-art diagnostic methods and consultation for clinicians who encounter patients with diagnosed or suspected *T. gondii* infection or toxoplasmosis. Today, this is only possible thanks to Jack S. Remington’s uninterrupted efforts and leadership during the last 4 decades in advancing the understanding, diagnosis, and treatment of the infection and diseases caused by *T. gondii*.

**Acknowledgments**

Jack’s well-known reputation for attention to detail and objectivity is portrayed in figure 1. The examples of the manuscripts and chapter shown here could easily be on its fourth or fifth draft. For his more than 700 publications, Jack’s vision and endless pursuit of perfection made it possible that such a draft could end up as a full publication in a journal or book. He has the legendary capacity to transform what appears chaotic, unrelated, and fragmented into what is logical, complete, and relevant. We, his fellows, owe him an immense debt of gratitude and respect and want to thank him for all the help and guidance he has provided us.

**References**


