Laboratory diagnosis of congenital toxoplasmosis

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Abstract

Recent studies have demonstrated that screening and treatment for toxoplasmosis during gestation results in a decrease of vertical transmission and clinical sequelae. Early treatment was associated with improved outcomes. Thus, laboratory methods should aim at the early identification of infants with congenital toxoplasmosis (CT). Diagnostic approaches should include, at least, detection of *Toxoplasma* IgG, IgM and IgA, and a comprehensive review of maternal history, including gestational age at which mother was infected and treatment. Here, we review laboratory methods for the diagnosis of CT, with emphasis on serological tools. A diagnostic algorithm that takes into account maternal history is presented.

**Keywords:** congenital infection, toxoplasmosis, serology
Introduction

In 1939 Wolf et al. reported for the first time that the intracellular protozoan parasite *Toxoplasma gondii* was a pathogen for humans and that it was capable of causing congenital disease as well (1, 2). Following this major discovery, it was promptly recognized that the clinical spectrum of fetuses, newborns and children congenitally infected with *T. gondii* could range widely from complete apparent normality to severe neurological and ocular disease, and even death (3). It is now well accepted that congenital toxoplasmosis (CT) has a worldwide distribution; recently, the global annual incidence of CT was estimated to be 190,000 cases (95% CI: 179,000 – 206,300), translating into an enormous disease burden of 1.20 million disability-adjusted life years (DALYs) (95% CI: 0.76–1.90) per annum (4). However, the morbidity and mortality associated with this disease is preventable, treatable, and reversible (3).

Effective anti-*Toxoplasma* treatment when instituted as early as in-utero during the gestational period has been shown to significantly decrease mother to child transmission as well as to improve clinical outcomes (5–10). Thus, it is imperative that laboratory tests employed for the diagnosis of CT be sensitive, specific and exhibit high predictive values in order to promptly identify fetuses and newborns that are likely to significantly benefit from treatment interventions. Here, we review serological tests currently available to clinicians for the diagnosis of CT. Additional methods, such polymerase chain reaction (PCR), histological stains, isolation of the parasite in mice, brain imaging studies and other general laboratory abnormalities associated with the disease, are mentioned but not reviewed in detail since they are beyond the scope of this review. In addition, the relevance of maternal history (e.g. gestational age at which...
mother was infected and whether the mother received anti-Toxoplasma treatment or not)
is incorporated in a diagnostic algorithm.

**General Considerations**

Congenital toxoplasmosis can occur when a woman acquires *T. gondii* infection for the first time during pregnancy or more rarely, shortly before conception. Infection of the fetus occurs when the parasite cross the hemato-placental barrier and reach the fetus.

The risk of transmission essentially varies with the gestational age and whether the mother receive treatment or not (5–10). The overall risk of transmission, in mothers who have been treated during gestation, is around 30%. However, it varies significantly with the gestational age at which the treated mother acquired the infection, from 15% at 13 weeks, 44% at 26 weeks to 71% at 36 weeks (10, 11). Less frequently, CT can also occur when women infected in the distant past and prior to gestation, reactivate their latent infection due to significant immunosuppression.

The diagnosis of CT can be confirmed or excluded more accurately when comprehensive clinical and laboratory information on the mother and her offspring is readily available to treating physicians. This information significantly affects the interpretation and pre-test probability of different laboratory tests (12, 13).

Ideally, it should be established first whether the mother is immunocompromised or immunocompetent and whether she belongs to one of three groups, 1) never infected with *Toxoplasma* and confirmed to remain seronegative one month after birth (no risk for CT), 2) chronically infected, mother acquired her infection prior to gestation (no risk for CT unless she is immunocompromised), 3) acutely infected, mother acquired her infection during gestation or within 3 months prior to gestation (at risk for CT). For group
3, it is important to establish (or estimate) the month during gestation at which maternal infection was acquired and whether the mother received anti-Toxoplasma treatment (if so, which drugs) since sensitivity and interpretation of laboratory tests can be largely affected by these variables (14). For instance, the sensitivity of serological test results in newborns is lower in those born to mothers who acquired their infection early in gestation and/or received anti-toxoplasmosis treatment during gestation than those born to mothers who acquired their infection late in gestation and/or did not receive treatment (14).

Information on the presence of clinical signs in the fetus and newborn may also be helpful in the interpretation and recommendations, for instance, regarding intervals for follow up testing after birth or indication for additional tests (e.g. Toxoplasma PCR).

**Principles and methods available for the diagnosis of congenital toxoplasmosis**

Several methods have been used for decades for the diagnosis of CT including detection of Toxoplasma-specific humoral immune responses, amplification of Toxoplasma DNA, identification of Toxoplasma-specific antigen in tissues and isolation of the parasite (Table 1) (15–17).

**Diagnosis of CT in the fetus**

During gestation, presence of the parasite in amniotic fluid (DNA amplification, microscopy or isolation of the organism), and/or fetal tissues (DNA amplification, antigen staining, microscopy or isolation of the organism) is diagnostic of CT (Table 1).

The most commonly used and accepted laboratory method for the diagnosis of CT during gestation is the use of PCR in amniotic fluid and a positive test result is diagnostic of CT.

**Diagnosis of CT in newborns and infants**
In the post-natal period, the gold standard to establish the diagnosis of CT is the persistence of *Toxoplasma* IgG by 12 months of age. Conversely, to rule out the diagnosis is the decrease of *Toxoplasma* IgG titers until its disappearance ≤ 12 months of age in the absence of treatment (Figure 1).

In the absence of comprehensive clinical history and previous *Toxoplasma* laboratory test results, diagnosis of CT after the first year of life is confounded by the possibility of the child acquiring infection in the post-natal period. For this reason, all reasonable efforts should be undertaken to diagnose or exclude CT during gestation or first year of life (infant period).

The most common laboratory method utilized worldwide for the diagnosis of CT in newborns and infants is serological detection of various isotypes of *Toxoplasma* antibodies in peripheral blood (serum). Although laboratories vary on the choice of specific method to detect *Toxoplasma*-specific antibodies, *Toxoplasma* IgG, IgM and IgA should always be tested since having the combination of IgM and IgA test results, in addition to the IgG, has greater sensitivity that either test alone (13, 15–17, 19).

*Toxoplasma* PCR testing in cerebrospinal fluid, peripheral blood, and urine can be another laboratory tool that can be used for early diagnosis of CT, being particularly helpful in regions where antenatal screening and treatment programs have not been implemented (18).

For *Toxoplasma* IgG detection, the dye test is the reference method and remains the gold standard. However, the dye test can only be performed in reference laboratories due to its dependence on the use of live parasites. Other methods more commonly used rely on ELISA and ELISA like assays, agglutination, indirect immunofluorescence.
Many ELISA and ELISA-like assays for detection of both *Toxoplasma* IgG and IgM use automated and closed systems that also allow sequential testing for the detection of IgG and IgM antibodies against other pathogens (17).

For *Toxoplasma* IgM detection, in addition to the same methods used for *Toxoplasma* IgG, the IgM immunosorbent agglutination assay (ISAGA) is used and is known for its overall higher sensitivity compared to ELISA and ELISA-like assays (e.g., 81.1% vs 64.8%) (20). The IgM ISAGA is considered the method of choice for the detection of *Toxoplasma* IgM in infants younger than 6 months of age (Table 2).

For *Toxoplasma* IgA detection, ELISA assays are mostly available. However, IgA ISAGA is also performed in few laboratories (20, 21).

Although the use of *Toxoplasma* IgE was initially reported as promising in some studies, it became subsequently obvious that it did not have any value to the combinatorial power of both IgM and IgA. (22, 23).

During the post-natal period, detection of *Toxoplasma* IgG is confounded by the fact that maternal IgG has been passively transferred across the placenta to the neonate. In addition, detection of *Toxoplasma* IgM and IgA in the neonate can also be contaminated with some maternal *Toxoplasma* IgM during the first 5 days of life and with maternal *Toxoplasma* IgA during the first 10 days of life. To overcome this challenge, methods to compare maternal and infant IgG, IgM, and IgA profiles have been developed such as Western blots. Western blots depict, several bands representing the binding between patient’s IgG, IgM, or IgA against various known *Toxoplasma* specific antigens. The principle is to compare these bands between mother’s and infant’s sera to detect, in case of congenital toxoplasmosis, autonomous synthesis of antibodies in the...
infant’s serum. This can be established by the presence of bands that are not present in the mother’s serum, or bands that have greater intensity in the infant when compared to their mother. Western blots have been shown to establish the diagnosis up to 3 months earlier than conventional serological methods (24). The sensitivity of Western blots in combination with conventional serological methods has been shown to be superior to western blots or conventional methods used alone. However, the interpretation of Western blots may be difficult and should not be performed after certain age due to false positive test results (e.g. for some kits false positive results are seen with Toxoplasma Western blot IgG and IgM after 1 and 3 months of life, respectively) (17, 25, 26).

Most non-reference laboratories can perform the Toxoplasma IgG, IgM and PCR tests. However, tests such as the IgM ISAGA, Western blots, and isolation are only performed in reference laboratories and have been validated in infants. In combination with results from conventional tests they yield a sensitivity that is greater than the sensitivity of each test alone (20, 23).

Few other methods have been described for the diagnosis of congenital toxoplasmosis. Enzyme-linked immunofiltration assay (ELIFA) is an alternative method to compare the immunological profile between mother and infant but it is performed only in very few laboratories (20). The interferon gamma released after T-cell stimulation by T. gondii antigens has be proven to be useful for the diagnosis of congenital toxoplasmosis but it is currently not commercially available (27). Detection and follow up of Toxoplasma IgG in oral fluid has also been used to monitor infants with suspected congenital toxoplasmosis and it is a promising diagnostic tool (28). In addition, brain imaging studies, and retinal exam can also exhibit findings that are highly suggestive of
the disease and in the absence of alternative etiologies and proper clinical context, they can be diagnostic (Table 1).

**Overall diagnostic approach**

Congenital toxoplasmosis can be diagnosed during gestation and/or after birth in the post-natal period. The diagnostic approach to newborns and infants varies significantly depending on whether their mothers were screened and treated during gestation and whether a diagnosis of fetal infection was attempted by amniocentesis.

Routine prenatal screening and treatment programs have only been implemented in few countries (Austria, Belgium, France, Norway, Uruguay, and some regions in Italy and Brazil). The clinicians managing newborns born in these regions benefit from having available information such as maternal serological and amniotic fluid PCR test results, precise gestational age at which the mother was infected and detailed anti-Toxoplasma treatment history.

In contrast, the vast majority of newborns worldwide including the United States are born in regions where such programs have not been implemented (23). Absence of, or incomplete prenatal screening and treatment, have been identified as an important risk factor for congenital toxoplasmosis (29).

**Differences in the diagnostic approach to CT according to presence or absence of maternal screening and treatment programs (Figure 1)**

Some important differences are observed in the approach, and performance and utilization of laboratory methods for the diagnoses of CT between regions with *Toxoplasma* screening and treatment programs during gestation and those without. The main objective of *Toxoplasma* maternal screening programs is to diagnose the acute
infection during gestation as early as possible. Women at risk (Toxoplasma seronegative) are identified during their first prenatal visit and followed at intervals that vary with the program (3). This approach allows prompt initiation of treatment of the mothers who seroconvert and of fetuses that get infected. This strategy has been shown to decrease mother to child transmission and severe disease and death in the offspring (9). As a result, fetuses and infants in these regions are diagnosed and treated much earlier than in regions where these programs have not been implemented. An unintended consequence of the screening/treatment approach is that laboratory methods such as serological and PCR tests are less sensitive in these regions (12, 23). However, pediatricians and clinicians managing these newborns have immediate access to maternal information and serum that are critical to the choice of laboratory methods for diagnosis of CT. For instance, they have available information that are known to influence choice and performance of laboratory tests such as precise knowledge about the gestational age at which the mother was infected and whether anti-Toxoplasma treatment was received, and if so, what regimen.

In regions where screening program is not implemented, critical information on the mother is usually not available. This lack of information often leads to unnecessary laboratory testing in infants who were not at risk of being infected and are later confirmed uninfected. In addition, treatment is delayed in newborns who are in fact infected (23). Absence of maternal treatment in regions without screening programs may explain why PCR is more commonly utilized for neonatal diagnosis in these regions since higher sensitivity in PCR assays is expected and has been observed in these regions (18, 23).
Similarities in the diagnostic approach to CT according to presence or absence of maternal screening and treatment programs (Figure 1)

Serological approach to diagnosis of CT in regions with or without these programs is similar once it has been established that the newborn belongs to one of these three categories: 1) CT is not likely because mother and newborn are *Toxoplasma* seronegative 2) CT is not likely because mother is chronically infected (the risk of CT is only present in newborns whose mothers are significantly immunocompromised), 3) CT is likely since mother was infected (or is suspected to have been infected) during gestation and/or newborn has clinical signs at birth.

Mothers who are *Toxoplasma* seronegative during gestation and confirmed to be so one month after birth, are not at risk for CT. Therefore, serological follow up in their newborns is not required. The reason to confirm the seronegativity status of the mother one month after birth is to rule out the rare possibility that mother could have been infected shortly before delivery.

Mothers who have been confirmed of having acquired the infection in the distant past and prior to pregnancy, are not at risk of CT unless the mother was actively immunosuppressed during gestation (e.g. mother with AIDS who reactivates her *Toxoplasma* infection or her CD4 count is below 200 cells/mm$^3$). Newborns born to these mothers will have detectable *Toxoplasma* IgG transferred passively from the mother and do not require serological follow up. However, in regions where maternal screening programs have not been implemented, certainty that mother has been infected in the distant past and prior to gestation is often impossible. Frequently, the first serum available has been obtained in the second trimester or later, or at birth. In these situations,
when the serological test results exhibit a positive Toxoplasma IgG and IgM, only reference laboratories such as the Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL http://www.pamf.org/Serology/) have serological assays (e.g. IgG Dye test, IgM ELISA, Avidity, differential agglutination (acetone [AC]-fixed versus formalin [HS]-fixed tachyzoites) test (AC/HS test), IgA and IgE ELISA assays) that can attempt to establish that the mother was infected in the distant past and prior to gestation \(^{(30, 31)}\).

Mothers who have been confirmed of having acquired the infection during pregnancy or shortly before gestation (e.g. within 3 months of conception) are at risk for CT. This risk can be reduced if treatment with spiramycin is initiated. In the United States, spiramycin is available at no cost only through by pursuing an investigational new drug (IND) application through the Food and Drug Administration (FDA). In regions where the screening programs have been implemented, the diagnosis of maternal infection acquired during gestation is ascertained by seroconversion. In regions without these programs, maternal serum is usually not available or only available from late in gestation or at birth, when clinical signs suggestive of congenital infection have been revealed in the fetus, newborn, or infant. With these late sera, only testing at references laboratories such as PAMF-TSL has the potential of determining whether mothers were likely to have been infected in the distant past and prior to gestation or during pregnancy.

The aim of serological testing in newborns born to these mothers, is to confirm, establish or exclude the diagnosis of CT. In newborns with positive amniotic fluid PCR tests results, serological testing and follow up are still recommended in order to further confirm the diagnosis of CT, and have additional data in cases where the possibility of a
false positive PCR test result is raised. In newborns with negative amniotic fluid PCR test results, or those in whom amniocentesis was not performed, serological testing and follow up is paramount as a potential diagnostic tool. The newborn serological panel comprised of *Toxoplasma* IgG, IgM ISAGA, and IgA should be performed regardless whether the amniotic fluid PCR test was performed and its results. The initial serum should be obtained after 10 days of life in order to avoid misleading results due to potential contamination with maternal blood. Follow up serum samples should be tested in parallel with the previous sample at one month, two months and then every two months (Figure 1).

Each newborn born to a mother who has been confirmed of or is suspected to have been infected during gestation, or shortly before conception, must followed up serologically until 12 months of age. The *Toxoplasma* IgG will decrease by half every month until its disappearance around month 6 or 7 in uninfected infants but will not disappear by 12 months of age in the infected infant. In some congenitally infected infants, treatment with pyrimethamine-sulphonamide combination can lead to negativization of the *Toxoplasma* IgG during follow up creating the false sense that the infant is not infected. However, in infected infants, discontinuation of the treatment is followed by a rebound in the *Toxoplasma* IgG. If IgG remains negative, assuming infant is capable of producing IgG, the diagnosis of CT is excluded.

**Future Directions**

The laboratory diagnosis of congenital toxoplasmosis has benefit from various principles and methods (Table 1). Future research should address cost and feasibility of detection of antibodies, DNA and live parasite in different platforms and body
compartments. For instance, simultaneous detection of multiple analytes in the same assay offer an attractive option for multiplex detection of *Toxoplasma* IgG, IgM and IgA, and of antibodies against other pathogens with capacity to cause congenital infection (32, 33). Use of platforms with multiplex capacity can address cost, with the additional benefit that could be extended to other infections. In addition the feasibility of testing for antibodies in body compartments beyond serum such as whole blood and saliva, can address cost and patient’s convenience (28). Lastly, public health authorities and national policies should address the need to fund and protect reference centers of excellence for the diagnosis and management of congenital infections since these infections may not be seen commonly in individual practices or medical centers but are a source of major morbidity and mortality to the fetus and newborns.
FIG 1 Congenital toxoplasmosis diagnostic algorithm for testing and monitoring infants according to whether maternal antenatal screening and treatment was performed (a) or not (b).

Cases in grey and white represent data and/or action before and after birth, respectively.


Dr. Montoya is originally from Cali, Colombia and completed his medical degree with honors at the Universidad del Valle. He trained in Internal Medicine at Tulane University in New Orleans. Following his residency, he completed his fellowship in Infectious Diseases at Stanford University in Palo Alto under the mentorship of Dr. Jack S. Remington. He is currently Professor in the Department of Medicine and Division of Infectious Diseases and Geographic Medicine at the Stanford University School of Medicine:

https://med.stanford.edu/profiles/Jose_Montoya. He is also the Director of the National Reference Laboratory for the Diagnosis and Management of Toxoplasmosis in the United States at the Palo Alto Medical Foundation in Palo Alto, California:

http://www.pamf.org/Serology/. He is also the founder of the Immunocompromised Host Service (Infectious Diseases) at Stanford University Medical Center. He was elected Fellow of the American College of Physicians (FACP) in recognition of commitment to the internal medicine community and Fellow of the Infectious Diseases Society of America” (FIDSA) for having achieved professional excellence in the field of Infectious Diseases. His research interests include toxoplasmosis, infectious complications in immunocompromised hosts and chronic unexplained illnesses likely triggered or aggravated by infection.

Christelle Pomares, M.D., PhD is an Associate Professor in Parasitology – Mycology Laboratory in the University Hospital
of Nice (France) and in the research team Inserm 1065 C3M, University of Nice Sophia Antipolis (France). She did her medical school in the School of Medicine in Nice Sophia Antipolis University (France) and her PhD. specialty of Communicable Diseases and Tropical Diseases in the Aix-Marseille University (France). She has been working on Toxoplasmosis since her fellowship with emphasis in serological diagnosis of *Toxoplasma* infection and its applicability to various patients’ populations. She is currently a Visiting Scholar at Stanford University Division of Infectious Diseases and Geographic Medicine (Stanford, CA, USA) working in the National Reference Laboratory for Toxoplasmosis in the United States directed by Pr. JG. Montoya.
The presence of only one of the criteria listed below is diagnostic of congenital toxoplasmosis:

- Presence of IgM and/or IgA at ≥ 10 days of life and/or during follow up samples
- In the newborn, presence of specific bands, or bands with higher intensity than maternal ones, for IgG and/or IgM and/or IgA western blot
- Persistence or increase in IgG titer without treatment ≤ 12 months of age

The diagnosis of congenital toxoplasmosis is excluded if:

- Absence of IgG titer without treatment is documented ≤ 12 months of age
- Testing for IgG, IgM, IgA detection at birth by western blot, or by conventional serologies at ≥ 10 days of life.
- If diagnosis is not made with this initial testing, follow up testing with IgG, IgM and IgA at one month and every two months thereafter is indicated

No infant follow up

Maternal infection has been acquired during pregnancy and amniotic fluid PCR is positive

TREATMENT

Serological confirmation (IgG, IgM, IgA) in order to further confirm diagnosis of CT and rule out the possibility of a false positive PCR test result

Testing for IgG, IgM, IgA detection at birth by western blot, or by conventional serologies at ≥ 10 days of life.

If diagnosis is not made with this initial testing, follow up testing with IgG, IgM and IgA at one month and every two months thereafter is indicated

No Congenital toxoplasmosis

TREATMENT

Maternal infection has been acquired during pregnancy and amniotic fluid PCR is negative or amniocentesis was not performed

The presence of only one of the criteria listed below is diagnostic of congenital toxoplasmosis:

- Presence of IgG plus IgM and/or IgA

The diagnosis of congenital toxoplasmosis is excluded if:

- Absence of IgG titer without treatment is documented ≤ 12 months of age
- Testing for IgG, IgM, IgA detection at birth by conventional serologies at ≥ 10 days of life.
- If diagnosis is not made with this initial testing, follow up testing with IgG, IgM and IgA at one month and every two months thereafter is indicated

No infant follow up

Congenital toxoplasmosis

TREATMENT

Suspicion of congenitally acquired infection and/or clinical signs at birth. Since maternal screening has not been performed during gestation, maternal serum at birth is required in order to perform parallel testing with newborn serum

Maternal serum is Toxoplasma seronegative at birth and it is confirmed to remain negative one month after birth, or maternal infection is confirmed to have been acquired prior to gestation

No infant follow up

Congenital toxoplasmosis

TREATMENT

Presence of IgG plus IgM and/or IgA

Testing for IgG, IgM, IgA detection at birth by conventional serologies at ≥ 10 days of life.

If diagnosis is not made with this initial testing

PCR on CSF, Whole Blood, Urine depending infant’s clinical signs

Negative

Positive

Congenital toxoplasmosis

TREATMENT

Follow up testing with IgG, IgM and IgA at one month and every two months thereafter is indicated

The presence of only one of the criteria listed below is diagnostic of congenital toxoplasmosis:

- Presence of IgG and/or IgA at ≥ 10 days of life and/or during follow up samples
- Persistence or increase in IgG titer without treatment ≤ 12 months of age

The diagnosis of congenital toxoplasmosis is excluded if:

- Absence of IgG titer without treatment is documented ≤ 12 months of age
- Testing for IgG, IgM, IgA detection at birth by western blot, or by conventional serologies at ≥ 10 days of life.
- If diagnosis is not made with this initial testing, follow up testing with IgG, IgM and IgA at one month and every two months thereafter is indicated

No infant follow up

Congenital toxoplasmosis

TREATMENT

No Congenital toxoplasmosis

No Treatment
<table>
<thead>
<tr>
<th>Principle</th>
<th>Detection</th>
<th>Platform</th>
<th>Diagnostic of Congenital Toxoplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxoplasma-specific humoral responses</strong></td>
<td>IgG, IgM, IgA</td>
<td>Dye test, ELISA and ELISA like assays, ISAGA, Immunofluorescence, agglutination</td>
<td>Positive IgM after 5 days of life and in the absence of blood transfusions. Positive IgA after 10 days of life. Persistence of Toxoplasma IgG beyond 1 year of age</td>
</tr>
<tr>
<td></td>
<td>IgG, IgM and IgA to specific Toxoplasma antigens</td>
<td>Western blots</td>
<td>Presence of specific bands only seen in the newborn or bands with higher intensity than maternal ones for IgG and/or IgM and/or IgA in a reference laboratory</td>
</tr>
<tr>
<td><strong>Toxoplasma nucleic acid amplification</strong></td>
<td>DNA</td>
<td>Polymerase chain reaction (PCR)</td>
<td>Positive result in any body fluid (e.g. amniotic fluid, cerebrospinal fluid *, peripheral blood, urine)</td>
</tr>
<tr>
<td><strong>Immunohistochemistry of Toxoplasma specific antigens in tissue</strong></td>
<td>Antigens</td>
<td>Immunoperoxidase</td>
<td>Positive result in any tissue (e.g. brain or other fetal tissue)</td>
</tr>
<tr>
<td><strong>Visualization by microscopy</strong></td>
<td>Visual identification of tachyzoites and/or cysts</td>
<td>Stains such as hematoxilin/eosin, Giemsa</td>
<td>Positive identification in a reference laboratory</td>
</tr>
<tr>
<td><strong>Isolation of Toxoplasma</strong></td>
<td>Whole live parasite</td>
<td>Inoculation in peritoneal cavity of mice</td>
<td>Detection of live cysts from any body fluid or tissue that has been inoculated in mice in a reference lab</td>
</tr>
<tr>
<td><strong>Brain imaging</strong></td>
<td>Brain calcifications, hydrocephaly, microcephaly</td>
<td>Ultrasound, computer tomography (CT), brain magnetic resonance (MRI)</td>
<td>Findings can be suggestive but are not diagnostic of CT since other etiologies may result in similar findings</td>
</tr>
<tr>
<td>Retinal exam</td>
<td>Inflammation in choroidal and retinal layers</td>
<td>Ophthalmological exam</td>
<td>Retinochoroidal lesions can be highly suggestive, or at times, diagnostic of congenital toxoplasmosis</td>
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</tbody>
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3

4  * In CSF, an extremely high level of protein (e.g. > 1000 mg/dl), presence of eosinophil and

5  detection of *Toxoplasma* IgM are also highly suggestive of congenital toxoplasmosis.

6
TABLE 2 Overview of sensitivity and specificity of *Toxoplasma* serological tests in the neonatal period.

<table>
<thead>
<tr>
<th>Principle of the test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM ELISA and ELISA-like assays</td>
<td>44% - 81%</td>
<td>88.8% - 100%</td>
<td>In two studies, sensitivity was found very low (below 30%)</td>
<td>(11, 12, 20, 34, 35)</td>
</tr>
<tr>
<td>IgM ISAGA</td>
<td>44% – 86.6%</td>
<td>77.7% - 100%</td>
<td>IgM ISAGA is the most sensitive test for IgM detection for newborn serology</td>
<td>(11, 20, 23, 36)</td>
</tr>
<tr>
<td>IgA ELISA</td>
<td>52% - 92.7%</td>
<td>64% - 100%</td>
<td>In one study, sensitivity was found very low (below 40%)</td>
<td>(12, 13, 35–37)</td>
</tr>
<tr>
<td>IgA ISAGA</td>
<td>52.9% - 72.5%</td>
<td>77.7% - 97.4%</td>
<td></td>
<td>(20, 21)</td>
</tr>
<tr>
<td>IgG Western blot</td>
<td>33% - 73.45%</td>
<td>96.2% - 100%</td>
<td></td>
<td>(25, 26, 38)</td>
</tr>
<tr>
<td>IgM Western blot</td>
<td>54.8% - 78.6%</td>
<td>94.74% - 100%</td>
<td></td>
<td>(20, 25, 26)</td>
</tr>
</tbody>
</table>

Combination of tests

<table>
<thead>
<tr>
<th>Combination of tests</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM ISAGA and IgA ELISA</td>
<td>73%</td>
<td>98%</td>
<td>(13)</td>
</tr>
<tr>
<td>IgG + IgM Western blot</td>
<td>86.44%</td>
<td>94.74%</td>
<td>(26)</td>
</tr>
<tr>
<td>IgM + IgA ISAGA and IgG + IgM Western blot</td>
<td>87.5%</td>
<td>81.4%</td>
<td>(20)</td>
</tr>
</tbody>
</table>