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Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii;* Approved Guideline

This document is intended to serve as a guide to aid in the interpretation of tests for the diagnosis of *Toxoplasma* infection.

A guideline for global application developed through the NCCLS consensus process.

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Abstract

NCCLS document M36-A—*Clinical Use and Interpretation of Serologic Tests for* Toxoplasma gondii; *Approved Guideline* is intended to aid laboratorians and physicians in determining the status of patients potentially infected with *Toxoplasma gondii*. Because *Toxoplasma* organisms are rarely detected in humans infected with the parasites, immunodiagnostic methods are used to indicate the presence of the infection by detecting *Toxoplasma*-specific antibodies or parasite material in body fluids. Clinical toxoplasmosis can be categorized into four groups: 1) acquired in the immunocompetent patient; 2) acquired or reactivated in the immunodeficient patient; 3) ocular; and 4) congenital. Methods of diagnosis and their interpretations differ for each clinical category. This guideline summarizes the current methods of choice to diagnose toxoplasmosis and discusses the challenges associated with serologic testing for *Toxoplasma*.

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Contents

Foreword

The purpose of this project is to update and inform laboratory scientists and physicians concerning the appropriate selection, performance, and interpretation of *T. gondii* serodiagnostic tests. This educational effort addresses the serology of *T. gondii*, with particular attention to optimal serum collection times and follow-up testing, performance characteristics, interpretation of results, limitations of testing, and types of available tests.

This guideline is needed, because there currently exists a great deal of potential for misapplication and misinterpretation of *T. gondii* serodiagnostic tests, i.e., interpretation of results for different patient populations, variability of test result reporting, and lack of mandatory expression of test results. The clinician is presented with the problem of determining if an infection is newly acquired, reactivated, or chronic. The laboratorian is faced with choosing tests from an array of commercially available kits for IgG and IgM antibody detection. In the absence of a fairly sophisticated knowledge of the subtleties of *Toxoplasma* serology, there exists a dangerous potential for the misuse, misapplication, and general misunderstanding of test results.

This guideline is intended for clinical laboratory scientists, clinicians, and manufacturers involved in the diagnosis of toxoplasmosis.

Key Words

Antibody detection, antigen detection, avidity, congenital toxoplasmosis, diagnosis, EIA, IFA, IgA, IgE, IgG, IgM, PCR, serodiagnosis, *Toxoplasma* diagnostic products, *Toxoplasma gondii*, toxoplasmosis

Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii;* Approved Guideline

1 Scope

This guideline provides the user with information about the biology of *Toxoplasma gondii;* the methods available for use in the laboratory diagnosis of human toxoplasmosis; the techniques that should be performed for specific clinical situations; the interpretation of the laboratory results; and the problems inherent in these methods.

2 Introduction

Individuals infected with the protozoan parasite, *Toxoplasma gondii*, generally show no detectable signs of infection and require no treatment. A small percentage of patients may require treatment, i.e., those with CNS toxoplasmosis or active ocular disease. However, if a woman becomes infected during pregnancy and the infection is passed to the fetus, the fetus may be catastrophically affected. These effects may be minimized or averted if *Toxoplasma* infection is diagnosed in a timely fashion and therapy instituted.

The diagnosis of toxoplasmosis generally relies on the detection of *Toxoplasma*-specific antibodies. Many laboratorians and clinicians are not familiar with the available diagnostic tools, because toxoplasmosis is not generally considered by most physicians to be a serious infection in persons with normal immune function. However, detection of primary infection in a pregnant woman with appropriate patient management is important to minimize the potential severe effects on the fetus. Also, knowledge of an individual's antibody status is necessary for clinical management if the patient is immunosuppressed or has lymphadenopathy. There are a variety of commercially available kits for the detection of *Toxoplasma* antibodies with a multitude of sensitivity and specificity rates, creating a wide range of choices for laboratorians. Even assuming that the test results obtained are valid, correct interpretation of the results may be problematic due to lack of knowledge by laboratorians and clinicians.

3 Definitions

Immunoglobulin class//Immunoglobulin isotype – A classification of immunoglobins based on antigenic and structural differences of the heavy (H) chain; **NOTE:** There are five classes: IgG, IgA, IgM, IgD, and IgE.

4 Background

4.1 Life Cycle

Toxoplasma gondii is a protozoan parasite that infects most species of warm-blooded animals, including humans. Members of the cat family (Felidae) are the only known definitive hosts for the sexual stages of *T. gondii* and thus are the main reservoirs of infection. The three stages of this obligate intracellular parasite are:

1) tachyzoites, which rapidly proliferate and destroy infected cells during acute infection;
2) bradyzoites, which slowly multiply in tissue cysts; and
3) sporozoites in oocysts.

Tachyzoites and bradyzoites occur in body tissues; oocysts are excreted in cat feces. After tissue cysts or oocysts are ingested by the cat, viable organisms are released and invade epithelial cells of the small intestine where they undergo an asexual cycle followed by a sexual cycle with the production of oocysts, which are then excreted. The unsporulated (i.e., uninfective) oocyst takes one to five days after excretion to become sporulated (infective). Although cats shed oocysts for only one to two weeks, large numbers may be shed, often exceeding 100,000 per gram of feces.[1] Oocysts can survive in the environment for several months or longer and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70 °C for 10 minutes. Cats become infected with *T. gondii* by carnivorism. Therefore, feral cats and domestic cats that are allowed to roam outside are much more likely to become infected than domestic cats that are confined indoors and fed only commercially prepared cat food.

Human infection may be acquired in several ways:

- ingestion of undercooked, infected meat containing *Toxoplasma* cysts;
- ingestion of the oocyst from fecally contaminated hands, food, and water;
- organ transplantation or blood transfusion;
- transplacental transmission; and
- accidental inoculation of tachyzoites.

The two major routes of transmission of *Toxoplasma* to humans are oral and congenital.[2] Risk behaviors include eating undercooked, infected meat or eating food that has been cross-contaminated with undercooked, infected meat; working outside in the dirt (gardening, yard work); changing the cat litter box; drinking contaminated water; and eating unwashed fruits and vegetables.[3] In humans, ingesting either the tissue cyst or the oocyst results in the rupture of the cyst wall, releasing organisms that invade the intestinal epithelium, disseminate throughout the body via blood cells, and multiply intracellularly. The host cell dies and releases the tachyzoites, which invade adjacent cells and continue the process. The tachyzoites transform into bradyzoites and form tissue cysts, most commonly in skeletal muscle, myocardium, and brain; these cysts may remain throughout the life of the host. Recrudescence of clinical disease may occur if the host becomes immunosuppressed.

4.2 Epidemiology

Serologic prevalence data indicate that toxoplasmosis is one of the most common infections of humans throughout the world. The prevalence of positive serologic titers increases with age. Infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions. This distribution is probably related to conditions favoring sporulation and survival of oocysts. Variations in the prevalence of infection between geographic areas and between population groups within the same locale are also probably due to differences in exposure.[4] High prevalence of infection in France has been related to a preference for eating raw or undercooked meat. However, high prevalence in Central America has been related to the frequency of stray cats in a climate favoring survival of oocysts. In the United States in 1967, prevalence rates of up to 30% were found along the seacoast, with rates of less than 1% in the Rocky Mountains and the desert Southwest. More recent data comparing antibody prevalence in U.S. military recruits in 1962 and 1989 indicated a one-third decrease in seropositivity. The overall seroprevalence in the United States as determined with specimens collected by the third National Health and Nutrition Examination Survey (NHANES III) between 1988 and 1994 was found to be 22.5%, with seroprevalence among women of childbearing age (15 to 45 years) of 15%.[5]

4.3 Clinical Conditions

Toxoplasmosis can be clinically categorized into four groups of patients:

1) acquired in the immunocompetent patient;
2) acquired or reactivated in the immunosuppressed or immunodeficient patient;

3) congenital; and
4) ocular.

Acquired infection with *Toxoplasma* in immunocompetent individuals is generally an asymptomatic infection. However, 10 to 20% of patients with acute infection may develop lymphadenopathy, most commonly in the cervical region. The clinical course is benign and self-limited; symptoms usually resolve within a few weeks to a few months.

Immunodeficient patients often have CNS disease but may have myocarditis, pneumonitis, or myositis. In patients with AIDS, toxoplasmic encephalitis is the most common cause of multiple intracerebral mass lesions and is thought to be due to reactivation of chronic infection. Toxoplasmosis in patients being treated with immunosuppressive drugs may be due to either newly acquired or reactivated latent infection.

Congenital toxoplasmosis results from passage of parasites from the mother to the fetus when she acquires a primary infection during pregnancy. The incidence and severity of congenital toxoplasmosis vary with the trimester during which infection is acquired. Because treatment of the mother reportedly may reduce the incidence of congenital infection, prompt and accurate diagnosis is extremely important. Most infants with subclinical infection at birth will subsequently develop signs or symptoms of congenital toxoplasmosis unless the infection is treated.[6]

Ocular *Toxoplasma* infection, an important cause of chorioretinitis in the United States, is often a result of congenital infection. Patients may be asymptomatic until the second or third decade of life, when lesions develop in the eye due to cyst rupture. Chorioretinitis is characteristically bilateral in patients with congenitally acquired infection, but more often unilateral in individuals with recently acquired *Toxoplasma* infection.

5 Methods of Diagnosis

5.1 Parasite Identification

Attempts to determine the presence of *Toxoplasma* organisms are usually unrewarding, have limited clinical value in most situations, and generally are not recommended. Only rarely can the diagnosis of toxoplasmosis be documented by the observation of parasites in patient specimens. Secretions, excretions, body fluids, and tissues are potential specimens for direct observation of parasites. Fluid specimens such as heparinized blood, cerebrospinal fluid, or bronchoalveolar lavage fluid should be centrifuged for 10 minutes at 2,000 rpm and the sediment smeared on a microscope slide. The slides should be air-dried, fixed in absolute methanol, and stained with Giemsa for microscopic examination. Tachyzoites may be observed as free organisms or within host cells such as leukocytes. Well-preserved tachyzoites are crescent-shaped and stain well, but degenerating organisms may be oval and stain poorly. Tissue imprints stained with Giemsa may reveal *Toxoplasma* cysts. Immunological techniques have been used to identify parasites in tissue sections or tissue cultures; fluorescein- or peroxidase-labeled antisera may be useful in detecting tachyzoites in tissue sections.

Parasites can be isolated with limited success by inoculating patient tissue or body fluids into either mice or tissue culture cells. Fresh tissue samples are ground with saline in a mortar and pestle and inoculated into the peritoneum of mice or directly into tissue culture flasks. The mice should be monitored for four to six weeks; if the organism is virulent for mice, the parasites can often be demonstrated in the peritoneal fluid after five to ten days. However, if the organism is relatively avirulent for mice, as is usually the case, the mice may not be killed by the infection. If they survive for six weeks, they should be bled for serologic testing. If antibodies are present, the mouse brain should be examined for the presence of *Toxoplasma* cysts. Inoculate additional mice with brain homogenate from the initially inoculated mice and observe and recheck after six weeks. *T. gondii* grows in a variety of tissue culture cells. A cytopathic effect may be detected on direct examination after 24 to 96 hours in culture. Giemsa staining may reveal

parasite structure, but parasitized cells may be difficult to detect. Immunofluorescence allows more sensitive detection of the organisms. The following procedure can be used for parasite isolation from amniotic fluids:

(1) Centrifuge a 10-mL sample of amniotic fluid at 1,000 x g and resuspend the sediment in 8 mL of minimum essential medium.

(2) Inoculate 1 mL into cover slip cultures of human embryonic fibroblast cell line such as MRC-5 in 24-well plates, incubate for 96 hours, with one change of medium at 24 hours.

(3) Fix the cultures with cold acetone and examine the cover slips by indirect immunofluorescence for the presence of *T. gondii*.

The use of tissue culture for isolation permits more rapid diagnosis than mouse inoculation but may be less sensitive; both methods can be useful for diagnosing congenital toxoplasmosis.

5.2 Molecular Detection

Application of polymerase chain reaction (PCR) technology to detect *Toxoplasma* DNA may be useful in situations for which knowledge of recent infection is essential. PCR detection of *Toxoplasma* in blood, urine, cerebrospinal fluid (CSF), amniotic fluid, ocular fluid, and bronchial alveolar lavage is a strong indicator of active infection, but the absence of *Toxoplasma* DNA does not exclude toxoplasmosis. PCR is especially valuable in determining fetal infection: PCR testing of amniotic fluid has greatly decreased the time required to determine the presence of parasites by mouse or tissue culture inoculation. Sensitivity rates of PCR appear to be dependent upon the clinical situation: high in amniotic fluid (congenital toxoplasmosis) but much lower in patients with reactivation of infection (HIV-positive individuals). There is also a potential for both false-positive and false-negative findings to occur. In the U.S., several commercial laboratories offer PCR testing, but none of these tests are FDA-cleared.

5.3 Antibody Detection

Because *Toxoplasma gondii* organisms are rarely detected in humans with toxoplasmosis, serologic examination is used to indicate the presence of the infection by detecting *Toxoplasma*-specific antibodies. In adults, antibodies of IgG, IgM, IgA, and IgE isotypes become detectable within 7 to 15 days post-infection. The presence of IgG antibodies indicates infection with the parasite at some unknown time. The presence or absence of IgM, IgA, and IgE antibodies may aid in determining the approximate time of primary infection.[7]

Currently, there is no single antibody detection test that can determine on the basis of a single specimen exactly when a patient was infected.[8] Many tests have been created and combinations of tests have been employed, but unless the patient is observed to seroconvert from negative to positive in two samples drawn after gestation, our ability to determine the time of primary infection is still not sufficient to rule out infection during pregnancy in some cases.

5.3.1 Ig/IgG Antibody Detection Tests

5.3.1.1 Sabin-Feldman Dye Test

The Sabin-Feldman dye test (DT) is generally considered the standard against which all other tests have been judged. Patient serum is incubated with live *T. gondii* tachyzoites, complement, and methylene blue dye. If the patient has antibodies to *Toxoplasma*, the cell wall of the parasite is lysed and cannot take up the dye. The DT measures primarily IgG antibodies that usually appear within one to two weeks of infection, reach peak titers in six to eight weeks, and gradually decline over many years. This test is

highly sensitive and specific, but an occasional false negative has been reported. The DT has been widely used to determine the serologic status of both humans and some animals. The requirement of live organisms with the high potential for laboratory infections has led to the replacement of the DT with other tests in most laboratories.

5.3.1.2 Indirect Hemagglutination Test (IHA)

In the IHA test for toxoplasmosis, patient serum is incubated with tanned red blood cells (RBC) sensitized with a soluble *Toxoplasma* antigen preparation. If the patient has antibodies to *Toxoplasma*, the RBC will agglutinate. The IHA test is simple to perform, inexpensive, applicable for humans and animals, and practical for testing large numbers of serum samples. Technical disadvantages of the IHA test are variations in RBC quality and in antigens. Additionally, the IHA test detects antibody later than does the DT and frequently does not detect congenital infections in newborns. Consequently, the IHA test may be used for epidemiological studies to determine incidence/prevalence but should not be used as a diagnostic test for individuals, especially pregnant women or newborns. Currently, one FDA-cleared kit is available in the United States.

5.3.1.3 Agglutination Tests

Direct Agglutination (DA): Sera are treated with 2-mercaptoethanol to reduce IgM antibodies and then are incubated with formalin-treated tachyzoites. Agglutination of parasites takes place if the patient has *Toxoplasma* antibodies. Results obtained with the modified DA test correlate well qualitatively with results of the DT and indirect fluorescent antibody (IFA) test. This DA test is simple to perform, is not species-specific, and can be used to test human or animal sera. The DA test is not available commercially in the United States but is available in Europe.

Latex Agglutination (LA): Patient serum without pretreatment is reacted with antigen-sensitized latex particles. If *Toxoplasma*-specific antibodies are present, agglutination takes place. Because titers compare favorably with the results of the DT and the IFA test, the LA test is useful for IgG screening purposes. The LA test is simple, easy to perform, and inexpensive, and can be used to test humans and animals. However, a small percentage of false-positive reactions attributed to nonspecific IgM reactions have occurred. The LA test is available commercially in the United States, Japan, and Europe.

Differential Agglutination (AC/HS): Comparison of agglutination reactions of acetone-treated (AC) tachyzoites with those of formalin-treated (HS) tachyzoites may be helpful to distinguish acute from chronic infection. Serum specimens from patients with recently acquired toxoplasmosis agglutinate both AC and HS parasite suspensions, but serum samples from patients with chronic infections have high titers of HS agglutination but low or negative AC titers. This assay is not available commercially.

5.3.1.4 Indirect Fluorescent Antibody Test (IFA)

The IFA test for toxoplasmosis uses whole, formalin-fixed tachyzoites that are air-dried on slides as antigen. Patient serum is incubated on the antigen slide, and a fluorescein-labeled antispecies antibody is added to visualize the reaction. The IFA test results compare well with those of the DT. Consequently, the IFA has been widely used as a replacement for the DT because it eliminates the necessity of handling live parasites. False-positive reactions may occur in individuals with antinuclear antibodies. The IFA test is the ideal assay procedure for a laboratory that screens less than ten serum samples a week for determination of immune status, but has the disadvantages of requiring technical expertise in fluorescence and subjective determination of results. FDA-cleared IFA test kits for human antibodies are available for purchase in the United States and other countries.

5.3.1.5 Enzyme Immunoassay (EIA)

EIAs or enzyme-linked immunosorbent assays (ELISA) are the most widely used tests for human toxoplasmosis screening in commercial laboratories in the United States. Soluble antigen is absorbed onto a matrix; patient serum is incubated with the antigen, followed by enzyme-labeled antihuman antibody (conjugate), and then substrate. The resulting color development is read on a spectrophotometer. Results of the EIA for IgG compare favorably with results of the DT and the IFA tests in sensitivity, but some false positives have been reported. With some EIA kits, heat inactivation of serum may cause false-positive reactions. The EIA has advantages over the IFA test with regard to objectivity in determining results, continuous line quantitation, and automation for testing large numbers of specimens. Currently, several FDA-cleared EIA kits are available in the U.S.; many others are available throughout the world.

5.3.1.6 IgG Avidity

A modified EIA-IgG assay determines the antigen-binding avidity of *Toxoplasma*-specific IgG antibodies and has been found to be most useful as an indicator of chronic infections, thus allowing acute infections to be ruled out with a high degree of certainty. Specimens are tested in duplicate; one is treated with urea to dissociate low-avidity antibodies. A ratio of the two end point titers is then calculated and expressed as a percentage. The presence of high-avidity IgG antibodies indicates that the infection occurred more than 12 weeks or more than 16 weeks previously, depending on which commercial kit was used. The presence of low-avidity IgG antibodies may be detected for up to 12 months postinfection and so is not a reliable indicator of recent infection in a pregnant woman. Several commercial kits are available outside of the U.S., but none have been FDA-cleared.

5.3.2 Tests for Detecting IgM Antibodies

5.3.2.1 IFA-IgM

The IFA-IgM test is performed as the IFA-IgG test with the substitution of an anti-IgM conjugate. In most patients, titers increase within one week of onset and generally revert to negative within six to nine months of infection. However, false-positive reactions caused by rheumatoid factor and false-negative reactions caused by *Toxoplasma*-specific IgG blockage were observed in IFA-IgM tests, leading to the recommendation that specimens should be treated prior to testing to obtain only the IgM fraction for testing that decreases the effects of these interfering factors. The IFA-IgM test is probably the least sensitive of all IgM detection procedures. A number of IFA-IgM kits are available commercially in the United States; some include materials for separating serum components before testing.

5.3.2.2 EIA-IgM

There are two major types of EIA-IgM tests in use: the capture-IgM EIA and the indirect EIA. The capture-IgM EIA uses anti-IgM as the first layer absorbed to the matrix, which when flooded with a patient's serum, captures only the patient's IgM antibodies. *Toxoplasma* antigen is added, followed by an enzyme-labeled anti-*Toxoplasma* antiserum and substrate. Alternatively, the *Toxoplasma* antigen may be enzyme-labeled, thus decreasing the test performance time. The capture system eliminates potential interference by IgG and other antibodies by removing them physically from the reaction. The capture EIA-IgM test improves detection of congenital infections: 73% of newborn infants with proven congenital *Toxoplasma* infections, as compared to 25% detected by the IFA-IgM test. Although the capture EIA system is more sensitive for serum samples from newborns, it may detect IgM antibody longer in acute acquired infections; therefore, determining the time of infection in pregnant women is often not possible with these assays. Many U.S. commercial companies market EIA-IgM kits, some the indirect EIA format with a serum pretreatment step, and others the capture-IgM format. Even with technical improvements, false-positive reactions may still occur (see Section 9.5).

5.3.2.3 IgM-Immunosorbent Agglutination (ISAGA)

The IgM ISAGA employs both the capture-IgM step of the EIA with the whole organism antigen of the DA test. If the patient has *Toxoplasma*-specific IgM antibodies, the organisms are agglutinated. It is more sensitive than the IFA-IgM and the EIA-IgM in the diagnosis of congenital infections. One drawback to the increased sensitivity is that IgM antibody is detected for a longer time by ISAGA than EIA; therefore, determining the time of infection in pregnant women is not possible with this assay. The ISAGA test kit is commercially available in Europe but not in the United States.

5.3.3 IgA Tests

Detection of *Toxoplasma*-specific IgA is an additional tool to indicate infection in infants. All infants suspected of congenital infection should be tested for both IgA and IgM antibodies. IgA antibodies are found more frequently than IgM antibodies in congenitally infected infants; some infants may have only IgA or only IgM, but not both. In adults with acute acquired infection, detection of IgA in the absence of IgM occurs rarely, but detection of both IgA and IgM antibodies may be further evidence for recent infection. Commercial IgA kits are available outside the U.S., but none are currently cleared by the FDA for use in the U.S.

5.3.4 IgE Tests

Detection of *Toxoplasma*-specific IgE antibodies has been reported to perhaps aid in determination of acute infection. IgE antibodies may persist for many months; however, the absence of detectable IgE antibodies does not exclude the possibility of acute infection, especially in congenital toxoplasmosis. Commercial kits are not available.

5.4 Antigen Detection

EIA tests for antigen detection have found circulating antigens present in 20 to 65% of human patients with acute acquired infection, 0 to 33% of congenital infections, and 16% of AIDS patients. Although simultaneous detection of *Toxoplasma*-specific IgM antibodies and circulating antigens would indicate recent infection more precisely than would detection of IgM antibodies alone, antigen detection techniques appear to lack sensitivity in human samples. No antigen detection tests are available commercially.

6 Safety Precautions

Because it is often impossible to know which clinical specimens contain infections materials, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

7 Specimen Collection and Handling

Most laboratorians rely on reagents in prepackaged diagnostic kits to test for anti-*Toxoplasma* antibodies. The manufacturers of diagnostic kits, by federal regulation, must establish the specimen type to be used in testing. Serum, plasma, CSF, and eye fluid specimens may all be tested for antibodies and antigen. CSF and eye fluid should be tested in parallel with a serum sample drawn on the same date. Users are advised to follow the manufacturer's package insert. Specimens may be stored for several days at 4 °C or frozen for longer storage. Specimens may be shipped at ambient temperature unless they will be in transit for more than a week or will be subjected to extremely hot temperatures. NCCLS documents H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture* and H18—*Procedures for the Handling and Processing of Blood Specimens* provide detailed information on specimen collection and handling.

For tests other than antibody detection, contact the laboratory for instructions before collecting specimens to ensure proper collection and handling.

8 Clinical Use of Immunodiagnostic Tests

The methods of diagnosis and their interpretations may differ for each clinical category. In general, the following questions may be asked and answered:

Questions	Tests to Use
Is the patient infected?	IgG assays
Is the infection recently acquired?	IgM, IgA assays
How recently was infection acquired?	IgG avidity, differential agglutination
Is there evidence of active infection?	PCR, culture, antigen detection

If determination of antibody status is the reason for testing, a single specimen is satisfactory; acute and convalescent specimens are not necessary. In situations where determining the time of infection is important, specimens drawn at least two weeks apart may or may not be useful. In the majority of cases, detection of a rising IgG or IgM titer is not possible, because the titers have already reached a plateau by the time the initial sample is drawn. If two specimens are to be compared, they should be tested together. Results from runs made at different times, in different laboratories, or with different procedures should not be compared quantitatively, only qualitatively as positive or negative.

8.1 Determination of Antibody Status

Four situations occur in which baseline information about whether an individual has antibodies to *Toxoplasma* would be useful:

1) in women of childbearing age tested prior to conception;

2) before initiation of immunosuppressive (particularly T-cell suppressive) therapy;

3) after initial determination of HIV-1 positive status; and

4) in organ donors prior to transplantation.

Screening one serum specimen with a sensitive test for IgG antibodies such as DT, IFA, or EIA would be sufficient. A negative test result would indicate that the patient has not been infected. A positive result of any degree would indicate infection with *T. gondii* at some undetermined time.

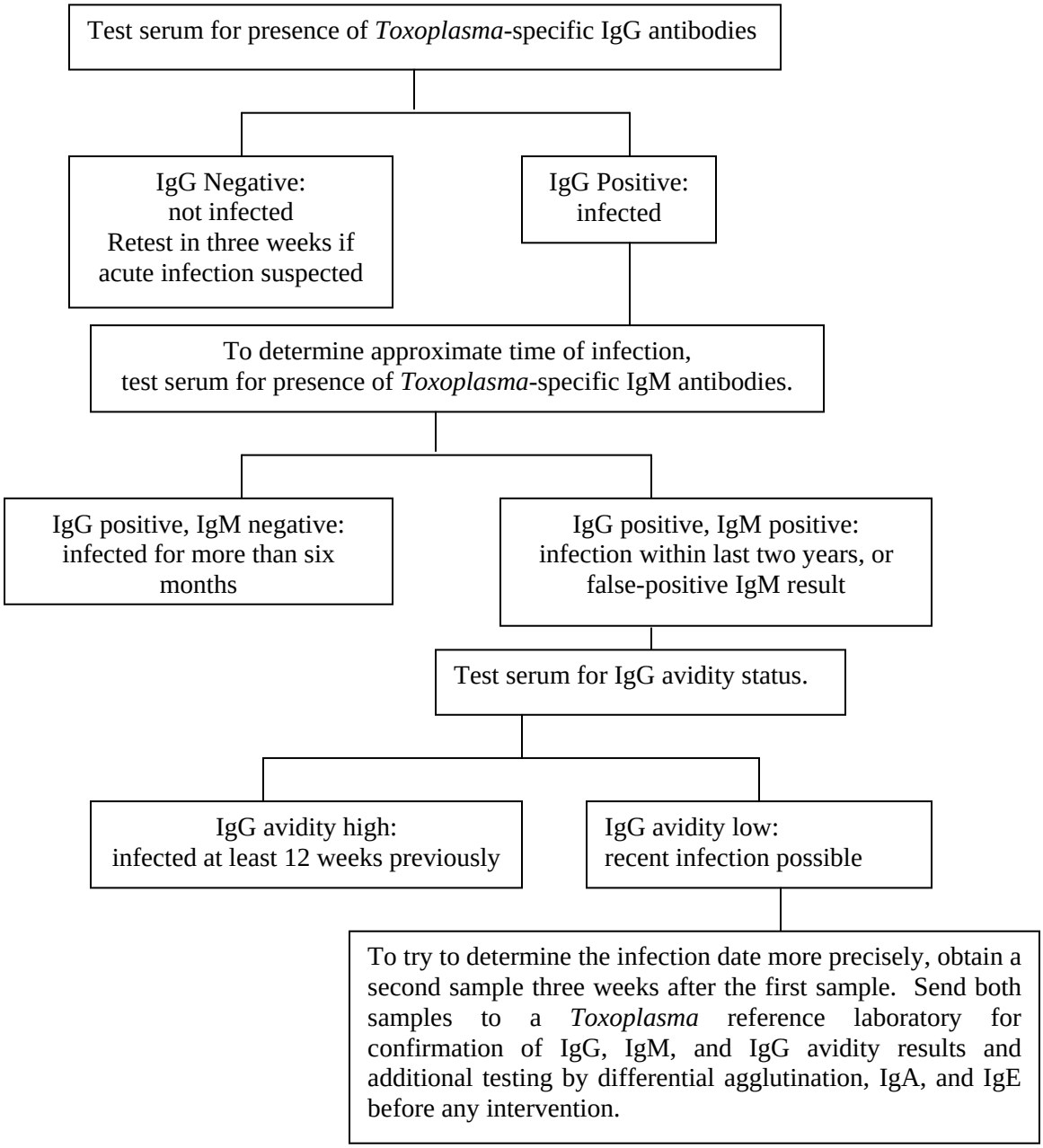

Figure 1. General Algorithm for Serological Testing of People Older Than One Year of Age

8.2 Diagnosis of Acute Acquired Infections

If a recently acquired infection is suspected, the patient's serum specimen should be tested for the presence of *Toxoplasma*-specific antibodies as shown in Figure 1 (above). A guide to the general interpretation of serology results is presented in Table 1. A negative result in DT, IFA-IgG, or EIA-IgG essentially excludes the diagnosis of recent *Toxoplasma* infection in an immunocompetent person. Demonstration of seroconversion from a negative to a positive titer or of more than a fourfold increase in titer confirms the diagnosis when specimens drawn several weeks apart are tested within the same test. However, such situations are rare, because specimens are usually drawn after titers have peaked, and are drawn too late to observe titer changes after initial infection. Results of an EIA for IgM and an IgG avidity assay can provide additional evidence for or against recent infection when IgG antibodies are present. A negative IgM test essentially rules out infection in recent months. A positive IgM titer combined with a positive IgG titer may be suggestive of recent infection; however, IgM antibodies have

been detected as long as 18 months after initial infection in some individuals. If the patient is pregnant, an IgG avidity test should then be performed. A high-avidity result in the first 12 to 16 weeks of pregnancy (time dependent upon the commercial test kit) essentially rules out an infection acquired during gestation. A low IgG avidity result should not be interpreted as indicating recent infection, because some individuals have persistent low IgG avidity for many months after infection. Suspected recent infection in a pregnant woman should be confirmed prior to intervention by having samples tested at a toxoplasmosis reference laboratory.[9] If the patient has clinical illness compatible with toxoplasmosis but the IgG titer is low, a follow-up titer two to three weeks later should show an increase in antibody titer if the illness is due to acute toxoplasmosis, assuming the host is not severely immunocompromised.

Table 1. Guide to General Interpretation of *Toxoplasma* Serology Results Obtained with Commercial Assays

IgG Result	*IgM Result*	*Report/Interpretation for humans, except infants*
Negative	Negative	No serological evidence of infection with *Toxoplasma*. If symptoms persist, obtain a new specimen three weeks later for testing.
Negative	Equivocal	Possible early acute infection or false-positive IgM reaction. Obtain a new specimen three weeks later for IgG and IgM testing. If results for the second specimen remain the same, the patient is probably not infected with *Toxoplasma.*
Negative	Positive	Possible recent infection or false-positive IgM result. Obtain a new specimen three weeks later for IgG and IgM testing. If results for the second specimen remain the same, the IgM reaction is probably a false positive.
Equivocal	Negative	Indeterminate: obtain a new specimen for testing or retest this specimen for IgG in a different assay.
Equivocal	Equivocal	Indeterminate: obtain a new specimen for both IgG and IgM testing.
Equivocal	Positive	Possible recent infection with *Toxoplasma.* Obtain a new specimen for IgG and IgM testing three weeks later. If results for the second specimen remain the same or if the IgG becomes positive, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Negative	Infected with *Toxoplasma* for usually more than six months.
Positive	Equivocal	Infected with *Toxoplasma*, but equivocal IgM results may be due to recent infection or false-positive IgM reaction. Obtain a second specimen three weeks later for testing. If results for the second specimen remain the same, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Positive	Possible recent infection. Send the specimen to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

8.3 Diagnosis of Congenital Infection

Diagnosis of congenital toxoplasmosis involves diagnosing recent infection in a pregnant woman and demonstrating infection in the fetus, or documenting infection in the newborn infant. Congenital toxoplasmosis occurs when a woman passes the infection to her fetus after acquiring primary infection during pregnancy, or more rarely, when a pregnant woman is immunocompromised and reactivation of a previously acquired infection allows transmission to the fetus. The rate of transmission of infection to the fetus ranges from 11% in the first trimester to 90% in the late third trimester, with an overall transmission rate of approximately 50%.

In France and Austria, the prevention, diagnosis, and treatment of congenital toxoplasmosis begin with mandatory serologic testing of all women prior to or soon after conception. This approach can serve as a model for managing an individual pregnant patient.

Immunocompetent women who have IgG antibody before conception are considered immune and so at very little risk for transmission of infection to the fetus. Women who are seronegative are considered at risk for infection and in France are tested monthly during pregnancy for IgG antibody. If a woman is first tested after conception and has *Toxoplasma*-specific IgG antibodies, IgM testing should be done. If the test results are IgM positive, avidity testing should be performed to determine if infection might have occurred during pregnancy. When the diagnosis of recent toxoplasmosis has been documented in a pregnant woman, she can be treated and the fetus can be tested for evidence of infection (see Figure 2). Amniotic fluid PCR at 18-weeks gestation is the recommended test of choice to establish the intrauterine diagnosis of congenital toxoplasmosis. However, amniotic fluid testing for *T. gondii* DNA by PCR is reported to have sensitivity rates ranging from 26 to 100% and specificity rates >90% for fetal infection. Test sensitivity and specificity are dependent upon the PCR targets and the skill of the testing laboratory. In addition, fetal ultrasound examinations should be performed every two to four weeks until delivery to look for several nonspecific signs of infection: cerebral or hepatic calcifications, hydrocephalus, hepatomegaly, or ascites.

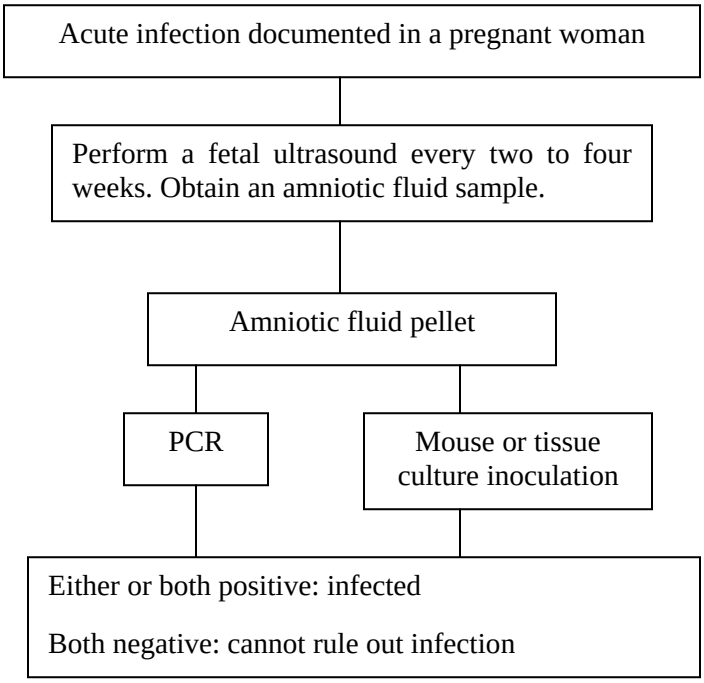

Figure 2. Algorithm for the Diagnosis of Antenatal Congenital Toxoplasmosis

If collected, fetal blood should be tested for *Toxoplasma*-specific IgG, IgM, and IgA antibodies. Clotted blood should be inoculated into mice or tissue culture cells to demonstrate parasitemia. Tests for nonspecific markers of infection should be performed; these include leukocytes, eosinophils, platelets, total IgM, gamma-glutamyltransferase, and lactate dehydrogenase. Most infected fetuses have one or more abnormal nonspecific tests, most commonly an elevated total IgM or an elevated gamma-glutamyltransferase level. Demonstrating *Toxoplasma*-specific IgM or IgA antibody in fetal fluid or isolating the parasite from fetal leukocytes or other tissues is a definitive diagnosis of fetal infection.

8.4 Diagnosis in the Newborn

Diagnosis of *Toxoplasma* infection in the newborn is made through a combination of serologic testing, parasite isolation, PCR, and nonspecific findings. The mother should be tested for *Toxoplasma* infection as indicated in Figure 1. The child's serum should be tested for total IgG and IgM antibody levels and *Toxoplasma*-specific IgG, IgM, and IgA antibodies. CSF should be analyzed for cells, glucose, protein, total IgG antibody, and *Toxoplasma*-specific IgG and IgM antibodies, and directly examined for *T. gondii* tachyzoites. An attempt should be made to isolate *T. gondii* from the placenta, amniotic fluid, and cord blood if the diagnosis has not already been established. *Toxoplasma* organisms have been isolated from 95% of the placentas of congenitally infected newborns when the mother has not been treated and from approximately 81% when the mother has been treated. However, *T. gondii* can be isolated from the placentas of uninfected newborns as well. A child suspected of having congenital toxoplasmosis should have a thorough general, neurologic, and ophthalmologic examination and a computed tomographic scan of the head (magnetic resonance imaging does not demonstrate calcifications). Because the diagnosis can take several months to confirm, clinicians may have to treat patients based upon early signs, symptoms, and serology, while awaiting definitive confirmation. Although the complexity of diagnosing congenital infection necessitates the use of multiple, costly laboratory tests, the benefit of early diagnosis and treatment and the cost of unnecessary treatment justify establishing the correct diagnosis.

Persistent or rising IgG antibody levels in the infant compared with the mother as measured by DT or IFA, and/or a positive *Toxoplasma*-specific IgM or IgA are diagnostic of congenital infection. Placental leak can occasionally lead to false-positive IgM or IgA measurements in the newborn. However, because the half-life of IgM is three to five days, repeat testing at one week should show a significant reduction in IgM antibody titer if the child is not infected. Repeat IgA testing should be done 10 days later to rule out maternal transfer. Passively transferred maternal IgG has a half-life of approximately one month. Maternal antibodies can be detected in the infant for several months after birth and have been reported up to one year of age depending upon how elevated the maternal titer is at delivery. The untreated, congenitally infected newborn will begin to produce *Toxoplasma*-specific IgG antibody within approximately three months. Treatment of the infected child may delay antibody production until nine months of age and on rare occasions, may prevent production altogether. Persistence of a positive IgG result at 12 months of life in the child is considered confirmatory of congenital infection. Demonstration of a decrease in "antibody load" (*Toxoplasma*-specific IgG antibody divided by total IgG) can be helpful in differentiating maternal antibody from fetal production. Demonstration by immunoblot in the newborn of serum antibodies that are directed against unique *Toxoplasma* epitopes not found in the mother's serum is evidence of congenital infection.

Although rarely performed, demonstration of IgM antibody or local *Toxoplasma*-specific IgG antibody production in CSF not contaminated with peripheral blood can help confirm the diagnosis of congenital toxoplasmosis. The calculation is made by dividing the *Toxoplasma*-specific antibody titer in the body fluid by the *Toxoplasma*-specific antibody titer in the serum and multiplying the result by the concentration of gamma globulin in serum divided by the concentration of gamma globulin in the body fluid. A result of four or greater corresponds to significant antibody production.

Use the following formula to calculate significant antibody production in CSF or ocular fluids:

$$\frac{\textit{Toxoplasma}\text{-specific antibody titer in CSF}}{\textit{Toxoplasma}\text{-specific antibody titer in the serum}} \text{ X } \frac{\text{concentration of gamma globulin in serum}}{\text{concentration of gamma globulin in CSF}}$$

A long-term prospective study is currently underway in the United States to define optimal therapeutic regimens for treatment of congenital toxoplasmosis.

8.5 Diagnosis of Ocular Infection

Many cases of *Toxoplasma* chorioretinitis probably result from congenital infection but may also occur during acute and chronic infection. In addition to demonstrating IgG antibody to *Toxoplasma* in the serum of a person with compatible eye lesions, the demonstration of local production of antibody and detection of parasite DNA in aqueous or vitreous humor have been used to document active ocular toxoplasmosis. When the formula described in Section 8.4 is used to calculate results obtained in eye fluids and with results obtained in serum, a value of 8 or greater suggests acute ocular toxoplasmosis. If the serum DT is greater than 1:1,000, it is usually not possible to calculate local antibody production.

8.6 Diagnosis in the Immunocompromised Host

A wide variety of immunosuppressed hosts, including patients with cancers, autoimmune diseases, or transplant recipients, has been described as having severe, often fatal, toxoplasmosis. The disease is most often related to reactivation of latent infection, and commonly involves the central nervous system, although a wide spectrum of clinical manifestations has been reported. Diagnosis can be very difficult in these patients as IgM antibody may not be detectable, and the presence of IgG antibody only confirms chronic infection. In the absence of serologic evidence of acute infection, diagnosis can be confirmed by histological or cytological demonstration of the organism replicating in tissue, or by isolation or identification of its nucleic acids in a site such as amniotic fluid, CSF, bronchoalveolar fluid, or placenta, in which the encysted organism would not be present as part of a latent infection.

Persons undergoing organ or bone marrow transplantation should have pretransplant testing for *Toxoplasma*-specific IgG antibodies to determine antibody status, because they are at risk for either acute acquired infection if they are seronegative prior to transplantation, or at risk for reactivation if they are seropositive before transplantation. Those with acute acquired infection often develop detectable *Toxoplasma*-specific IgG and IgM antibodies, while those with reactivation may or may not have an increase or decrease in IgG antibodies, but usually do not have a detectable *Toxoplasma*-specific IgM response. Seronegative transplant recipients of hearts from seropositive donors can develop toxoplasmic myocarditis that mimics organ rejection.

Toxoplasmic encephalitis was the most frequent CNS opportunistic infection of AIDS patients prior to use of highly active antiretroviral therapies (HAART) and is uniformly fatal if untreated. It is recommended that all HIV-infected persons be tested for *Toxoplasma*-specific IgG antibodies soon after the diagnosis of HIV infection to detect latent infection. Most AIDS patients with toxoplasmic encephalitis have demonstrable IgG antibodies to *T. gondii*. However, approximately 3% of AIDS patients with toxoplasmic encephalitis do not have *Toxoplasma*-specific antibody in their serum. Local production of *Toxoplasma*-specific IgG antibody in CSF has been demonstrated in persons with AIDS with toxoplasmic encephalitis. When the formula described in Section 8.4 is used, a result of greater than 1 corresponds to significant antibody production.

8.7 General Interpretation of Test Results

See Table 1.

9 Challenges Associated with Immunodiagnostic Testing

9.1 Commercial Kits

Commercial kits for agglutination, IFA, and EIA antibody detection tests are available worldwide. Because of difficulties in obtaining specimens from clinically documented cases of toxoplasmosis, commercial kit sensitivity and specificity generally is based on comparison of results obtained with another kit rather than on documented case specimens. For each kit, the user should review the published literature and the manufacturer's package insert for information on the kit's sensitivity and specificity rates.

The U.S. Medical Device Amendments of 1976 to the Federal Food, Drug, and Cosmetic Act require the FDA to regulate medical devices (i.e., *in vitro* diagnostic kits) that are intended for human use. Manufacturers and potential distributors are required to notify the FDA that they intend to offer a kit for sale and submit data to support performance claims. However, product clearance by the FDA does not guarantee test performance. The U.S. FDA approves the commercial distribution of tests in the U.S. after the manufacturer demonstrates reasonable assurance of safety and effectiveness in premarket approval (PMA) or after demonstrating the test is substantially equivalent to a previously cleared test [510(k)]. The FDA does no laboratory testing to validate these performance claims, nor is there any validation of lot-to-lot performance after clearance.

The most accurate means of comparing kits is to compare test results of all kits with identical samples. To achieve this goal, the Centers for Disease Control and Prevention (CDC) created a *Toxoplasma* Serum Panel, which is now available for purchase by manufacturers. The panel includes samples from *Toxoplasma* IgG-, IgM-, and/or IgA-positive patients as well as *Toxoplasma*-antibody-negative individuals. All new applications submitted to the FDA for *Toxoplasma in vitro* diagnostic devices must include test results of the new kit with this panel. The test results must also be included in the package insert. Kits cleared through this process prior to 1999 are not currently required to include the CDC panel data. Consequently, although there now exists a means for the end user to directly compare kits prior to performing an in-house evaluation, it will take many years before all *Toxoplasma* antibody detection kits will include this data. For further information about purchasing the panel, contact:

Reference Immunodiagnostic Laboratory, BDB
Division of Parasitic Diseases, NCID
Center for Disease Control, MS F-36
4770 Buford Highway
Atlanta, GA 30341 USA
Phone: 770-488-4431

In-house evaluation of kits must be performed when the decision is made to initiate serologic testing for *Toxoplasma*, to change from one assay to another, or when reagent lots are changed. A laboratory currently performing an assay should create and maintain a serum specimen bank of both *Toxoplasma*-negative and *Toxoplasma*-positive samples for use in future test evaluations. See NCCLS document I/LA21—*Clinical Evaluation of Immunoassays* for procedures to compare kits and NCCLS document I/LA18— *Specifications for Immunological Testing for Infectious Diseases* for additional information.

9.2 Choosing Tests

When the decision is made to institute *Toxoplasma* immunodiagnostic testing or change assays, several factors should be taken into consideration: the volume of tests requested, why tests are requested, the ease of use and adaptability to one's laboratory situation, and what data are available on the kit performance.

If one has a small volume of test requests and currently performs immunofluorescence tests, the IFA-IgG test may be more cost-effective than an EIA. Latex agglutination assays are economical and take little time to perform, but positive results need to be confirmed by a more specific assay. If one has a high volume of test requests, EIA tests may be automated and would probably be more suitable.

If tests are requested as part of a routine screening protocol for HIV-positive patients, for patients prior to transplant, or for women prior to conception, a sensitive IgG assay is sufficient. If a facility's patient population is mostly pregnant women, it might be useful to also have the capability to do IgM testing. However, dependent upon volume of test requests, it might be more efficient and cost-effective to send IgM requests to another laboratory that maintains more technical experience with an IgM test due to higher volume.

When determining which test to purchase, collect as much information as possible about the test. Thoroughly review the kit insert for data presented by the manufacturer. Review the published literature for articles about the kit, and compare its test parameters with those of other kits. Ask the sales representative for phone numbers of other clients who purchase that kit, call them, and ask their opinions about the kit. Ask the sales representative for a demonstration and/or sample kit to test in-house. Choose what appear to be the top two or three kits and evaluate them with the same samples before making a final decision.

9.3 Nonstandardized Use of Tests

The U.S. FDA has cleared NONE of the commercially available kits for *Toxoplasma* for use in testing fetal, newborn, or infant serum specimens, or CSF and ocular fluids from any source. To perform testing on these or other special groups of specimens, in-house test parameters of sensitivity and specificity must be established to allow for test result interpretation.

9.4 Nonstandardized Reporting of Results

Test results obtained with *Toxoplasma* kits are not standardized, due to the use of different antigen preparations, test procedures, and result nomenclature, making comparison of results very difficult. Results may be stated in international units, as an index (specific to each kit), as an optical density value (specific to each kit), or as a geometric mean titer. Because no uniform method of expressing results is used, particularly in the EIA kits, results obtained with two kits on the same **sample** may only be compared as positive (*Toxoplasma* antibody detected) or negative (not detected). For a direct comparison of results between paired specimens, both samples must be tested by the same test kit within the same test run.

The international standard reference serum for *Toxoplasma* distributed by the World Health Organization was originally evaluated for use in the DT test and is available from:

National Institute of Biological Standards and Control
Blanche Lane
South Mimms
Potters Bar, Hertfordshire EN6 3QG
United Kingdom

9.5 False-Positive Reactions

Many causes of false-positive reactions have been described: serum inactivation at 56 °C; antinuclear antibodies; rheumatoid factor (RF); competition between isotypes for antigen-binding sites on the solid phase; naturally occurring antibodies; and several reactions involving the Fc portion of immunoglobulins. The use of absorbents and/or use of the capture format (anti-IgM bound to the matrix) instead of the indirect test format (antigen bound to the matrix) eliminated the majority of problems with RF and isotype competition for binding sites in IgM tests. However, reports of additional causes of false-positivity continue to appear.

After becoming aware of inaccurate *Toxoplasma* IgM test results from clinical reference laboratories, proficiency testing surveys, CDC, and the scientific literature, the FDA sponsored an evaluation of commercial IgM kits. The results confirmed other published reports of specificity problems with several kits. As a result, the FDA issued a Public Health Advisory in July 1997, entitled "Limitations of *Toxoplasma* IgM Commercial Test Kits." Physicians were advised that the "result from any one *Toxoplasma* IgM commercial test kit should not be used as the sole determinant of recent *Toxoplasma* infection when screening a pregnant patient. Because these tests can have false-positive results, reliance on a single test result could lead to misdiagnosis, resulting in unnecessary treatment of the patient and/or termination of the pregnancy." Since the advisory was issued, the two kits found to be the least specific have been taken off the market.

Laboratorians and physicians need to remain aware of the possibility that an IgM reaction may or may not be a true positive. Guidelines for interpretation of IgG and IgM serology results are presented in Table 1.

9.6 Quality Assurance/Quality Control

The College of American Pathologists (325 Waukegan Rd., Northfield, IL 60093, http://www.cap.org, phone 800-323-4040 or 847-832-7000) offers the only organized proficiency testing (PT) program for *Toxoplasma* in the United States at the time of this printing. One sample is sent twice a year as part of the VR3 Virology Ab Detection Survey and so is insufficient to be a true PT program. However, the data resulting from this survey can give the laboratorian an idea of which assays are widely used and how well the results of laboratories compared with each specimen.

Under the U.S. Clinical Laboratory Improvement Act (CLIA) guidelines, all laboratories performing tests for which there is not a sufficient external PT program must institute an internal PT program. This must include the blinded testing by all technical personnel of at least five samples twice a year in each *Toxoplasma* assay. The results are submitted to the technical supervisor, compared to the expected results, approved/disapproved, and documented in the laboratory quality assurance records. Specimens chosen for this purpose should have well-documented results with a known range of reactivity. (See NCCLS document C24—*Statistical Quality Control for Quantitative Measurements: Principles and Definitions* and NCCLS document I/LA18—*Specifications for Immunological Testing for Infectious Diseases.*)

9.7 Reference Laboratories

Because of the potential difficulties involved in the diagnosis of toxoplasmosis, it is recommended that specimens from patients whose initial IgG and IgM results indicate possible recent infection be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis. Currently in the U.S., only two reference laboratories[a] offer the IgG avidity test, although this test is commonly available in Europe.[8] Other esoteric tests described in this standard have also been useful in distinguishing recently acquired infections.[9,10] In all cases, the results of laboratory tests indicating recent infection should be carefully interpreted by physicians with experience in the diagnosis of toxoplasmosis.[9]

[a] Focus Technologies, Cypress, CA, and Toxoplasma Serology Lab, Palo Alto Research Foundation, Palo Alto, CA

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NCCLS consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

M36-P: *Clinical Use and Interpretation of Serologic Tests for* Toxoplasma gondii; *Proposed Guideline*

Section 3.1 (now Section 4.1), Life Cycle

1. The third paragraph in Section 3.1 discusses dissemination throughout the body via blood lymphocytes. Change this to possibly read "blood cells" since it can also go into polys, etc.

- **This has been revised as suggested.**

Section 4.1 (now Section 5.1), Parasite Identification

2. In Section 4.1, second paragraph, the sentence reads, "If cysts are not observed, the murine host may not have been the ideal host." This statement has not been proven.

- **This sentence has been deleted.**

Section 4.2.1.5 (now Section 5.3.1.5), Enzyme Immunoassay (EIA)

3. The Abbott IMX package insert (April 1997, page 5) states heat-treated serum may be used.

- **The sentence referring to heat-treated serum has been revised.**

Section 4.2.1.6 (now Section 5.3.1.6), IgG Avidity

4. According to our experience, it is advisable to perform the toxoplasmosis serology in the period between the eighth and twelfth week of gestation in order to get the best conclusions concerning the IgG avidity test and the meaning of the presence of IgM antibodies.

- **Pregnant women, if not infected at conception, are at risk for infection with *T. gondii* during the entire pregnancy. Ideally, women at high risk for *Toxoplasma* infection should be tested immediately before conception and if not infected, then retested once a month during pregnancy to determine when infection, as indicated by seroconversion, occurs. However, in areas with moderate or low seroprevalence and low incidence, this approach is not recommended.**

Section 4.2.3 (now Section 5.3.3), IgA Tests

5. Explain what the P30 antigen is.

- **The reference to the P30 antigen in this section has been removed.**

Section 4.4 (now Section 5.2), Molecular Detection

6. Are there any PCR kits that are FDA approved? If not, state it here.

- **Text has been added as suggested.**

7. For PCR detection, add the word, "urine."

- **This has been added as suggested.**

Section 7 (now Section 8), Clinical Use of Immunodiagnostic Tests

8. It is not necessary to determine immune status. Suggest changing to read antibody status.

- **This change has been made as suggested.**

9. This section should include "quantitative result reporting" such as titre or International Units. This will aid clinicians to assess immune status.

- **Clinicians should not rely on individual quantitative test results, because they cannot be correlated to clinical status or time of infection.**

Section 7.1 (now Section 8.1), Determination of Antibody Status

10. Include all transplant patients and donors.

- **This has been added as suggested to Sections 8.1 and 8.6.**

Figure 1, General Algorithm for Serological Testing of People Older Than One Year of Age

11. Change "IgG Positive: infected" to read "IgG Positive, current or previous exposure." They may not be infected.

- **The subcommittee disagrees with this suggested change. The antibody-positive patient is infected.**

Table 1, Guide to General Interpretation of *Toxoplasma* Serology Results Obtained with Commercial Assays

12. The following text is suggested to be added to Table 1, Report/Interpretations:

    a) Negative-Negative – If symptoms persist, submit a new specimen within three weeks.

    - **A sentence has been added as suggested.**

    b) Equivocal-Equivocal – If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference lab with experience in the diagnosis of toxoplasmosis.

    - **The results of the second specimen should be interpreted as stated in Table 1.**

    c) Positive-Negative – Add to the beginning of the sentence, "It appears that the patient has been previously…"

    - **This suggested text is not consistent with the table style.**

    d) Positive-Equivocal – Change to read, "It appears the patient has been previously infected with Toxoplasma for probably more than one year and residual IgM antibodies still exist."

    - **This suggested text is not consistent with the table style.**

Section 7.4 (now Section 8.4), Diagnosis in the Newborn

13. Would it be best to give any example in mathematical form of the calculation referred to in Section 7.4?

- **This has been added as suggested.**

14. For increased IgA, include a sentence to repeat testing in 10 days to rule out maternal transfer.

- **A sentence has been added as suggested.**

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

M36-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X EP9 EP12 I/LA18 I/LA21 M15			GP29			X M29

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M36-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
X H4 I/LA18 I/LA21		X H4 H18 M15	H18 M15	H18	X I/LA18 I/LA21 M15	X M15	X M15	H18 I/LA21

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Related NCCLS Publications*

EP9-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods or devices, and for the design of a method comparison experiment using split patient samples and data analysis.

EP12-A **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline (2002).** This document contains a protocol that optimizes the experimental design for the evaluation of qualitative tests, to better measure performance and provide a structured data analysis.

GP29-A **Assessment of Laboratory Tests When Proficiency Testing is Not Available; Approved Guideline (2002).** This document offers methods to assess test performance when proficiency testing (PT) is not available; these methods include examples with statistical analyses. This document is intended for use by laboratory managers and testing personnel in traditional clinical laboratories as well as in point-of-care and bedside testing environments.

H4-A4 **Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved Standard—Fourth Edition (1999).** A consolidation of H4-A3 and H14-A2, this standard provides detailed descriptions and explanations of proper collection techniques, as well as hazards to patients from inappropriate specimen collection by skin puncture procedures.

H18-A2 **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Second Edition (1999).** This guideline addresses multiple factors associated with handling and processing specimens, as well as factors that can introduce imprecision or systematic bias into results.

I/LA18-A2 **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline—Second Edition (2001).** This guideline outlines specimen requirements; performance criteria; algorithms for the potential use of sequential or duplicate testing; recommendations for intermethod comparisons of immunological test kits for detecting infectious diseases; and specifications for development of reference materials.

I/LA21-A **Clinical Evaluation of Immunoassays; Approved Guideline (2002).** This guideline will offer recommendations on designing trials that are appropriate for evaluating both the safety and effectiveness of immunoassays. It will be a valuable resource in determining the necessary steps in designing an evaluation for new methods, new applications for existing methods, or variations on existing methods.

M15-A **Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline (2000).** This document contains guidelines for specimen collection, blood film preparation, and staining procedures. Recommendations for optimum timing of specimen collection to assist laboratories in detecting, identifying, and reporting certain parasites are also included.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2002).** Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmissions of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

NOTES

NOTES

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