CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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M36-A

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 Clinical and Laboratory Standards Institute consensus process.

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For additional information on committee participation or to submit comments, contact CLSI.

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

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Abstract

CLSI 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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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;

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.

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