CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Toxoplasmosis, IgG and IgM
- Improved Assays –

Contact: J. Matthew Groeller, 717.851.1416
Operations Manager, Clinical Pathology, CPAL

Dr. Jennifer Thebo, 717.851.3210
Director, Clinical Pathology, CPAL

Affected Tests:

Mnemonics:	Toxo IgG	Toxo IgM
Test Name:	Toxoplasmosis IgG	Toxoplasmosis IgM
Test Number:	3001410	3001420
Specimen:	0.5 mL serum, 2-8^{0}C up to 7 days, -20^{0}C or colder up to 6 months	

Effective Date: Testing start date anticipated as May 1, 2014.

Performed: Monday, Wednesday, and Friday

Reference Range: Negative

Method Change:
CPAL will be implementing improved assays for Toxoplasmosis IgG and IgM. The new assays provide for longer sample stability (7 days vs 48 hrs refrigerated). Please note that reference intervals will change when implemented as shown under "Results Interpretation" below.

Background:
Toxoplasmosis is a quite widespread infectious disease caused by an intracellular protozoan parasite, called *Toxoplasma gondii*. The disease, affecting both man and warm-blooded animals, can be transmitted by ingestion of food infected or contaminated by oocysts; direct contagion from domestic animals; or transplacental infection to newborn. Transmission of *Toxoplasma* through blood transfusions or organ transplantation has also been reported in the literature. In the normal adult population, toxoplasmosis has a generally benign course, being largely asymptomatic; sometimes mildly symptomatic (headache, sore throat, asthenia); or in rare cases accompanied by lymphadenitis. The prevalence of positive serological tests increases with age, indicating past exposure. Cell-mediated immunity is generally involved in protecting from parasite infection. As a consequence, a symptomatic course is generally more frequent in immunocompromised subjects such as patients undergoing immunosuppressive therapy or patients with acquired immunodeficiency syndrome. If the infection occurs in pregnant women, toxoplasmosis can cause a threat to the fetus with possible spontaneous

abortion, prematurity or stillbirth, as the pathogen can be transmitted to the fetus via the placenta. The fetus whose mother is exposed to *Toxoplasma* infection during the first trimester of pregnancy develops severe lesions to the central nervous system that generally lead to fetal demise. *Toxoplasma* infection acquired during the second trimester may cause hydrocephalus, mental and psychomotor retardation, blindness, and cerebral calcifications. *Toxoplasma* infection, however, is most common during the third trimester, causing retinochoroiditis and other ocular lesions, lesions to the central nervous system, and latent asymptomatic infection which may eventually develop into full-blown disease. Because of the diversity or absence of symptoms, the detection of *Toxoplasma* infection during pregnancy has to be based on maternal serology rather than on clinical findings. The serological diagnosis of acute toxoplasmosis allows adequate treatment which reduces the risks of the disease both in immune-compromised patients and in pregnant women.

Specific IgG antibodies to *Toxoplasma* rise gradually and peak two to five months after the onset of infection. Therefore, the presence of IgG is useful in distinguishing subjects who have acquired the disease from those who have not. This is particularly important to identify susceptible women of child-bearing age. Specific IgM antibodies to *Toxoplasma* develop two to four weeks after the onset of infection, rapidly increasing and gradually declining thereafter, generally disappearing in three to nine months. The presence of IgM in the absence of IgG or in the presence of low IgG levels is generally indicative of acute toxoplasmosis.

Method Principle:

The method for qualitative determination of **IgG** antibodies to *Toxoplasma gondii* (anti-Toxo IgG) is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the analyzer. The principal components of the test are magnetic particles (solid phase) coated with *Toxoplasma gondii* and a conjugate of mouse monoclonal antibodies to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, *Toxoplasma gondii* antibodies present in diluted calibrators, samples, or controls bind to the solid phase. During the second incubation, the monoclonal antibody conjugate reacts with anti-Toxo IgG that is already bound to the solid phase. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and therefore, the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of anti-Toxo IgG in calibrators, samples, or controls.

The method for qualitative determination of specific **IgM** to *Toxoplasma gondii* is an antibody capture chemiluminescence immunoassay (CLIA). IgG to human IgM (mouse, monoclonal) is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody to Toxoplasma gondii major surface antigen (SAG1) is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, IgM antibodies present in calibrators, samples, or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with *Toxoplasma gondii* antigen previously added and the immune complex thus formed reacts with IgM already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of *Toxoplasma gondii* IgM concentration present in calibrators, samples, or controls.

Results Interpretation:
Both Toxo IgG and IgM are interpreted according to index values shown in tables 1 and 2 respectively.

Table 1: Interpretation of Toxo IgG

Index	Results	Interpretation
< 7.2 IU/mL	Negative	Absence of detectable *Toxoplasma gondii* IgG antibodies. A negative result does not rule out acute infection. The test usually scores negative in infected patients during the incubation period and the early stages of infection. If exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested one or two weeks later.
≥ 7.2 - < 8.8 IU/mL	Equivocal	The equivocal sample should be repeat tested. In case the result remains in this range after repeat testing, a second sample should be collected and tested no less than one or two weeks later.
≥ 8.8 IU/mL	Positive	Presence of detectable *Toxoplasma gondii* IgG antibodies. A positive result generally indicates either recent or past exposure to the pathogen. If IgG test scores positive in the presence of IgM antibodies, recent infection may be postulated. If IgG test scored positive in the absence of IgM antibodies, past infection may be postulated.

Note - *The magnitude of the measured result is not indicative of the amount of antibody present. The concentrations of anti-Toxoplasma gondii IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.*

Table 2: Interpretation of Toxo IgM

Index	Results	Interpretation
< 8.0 AU/mL	Negative	Absence of detectable *Toxoplasma gondii* IgM antibodies. A negative result does not always rule out acute toxoplasmosis, because the infection may be in its very early stage and the patient has not developed *Toxoplasma gondii* specific IgM. If exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested three weeks later.
≥ 8.0 AU/mL and < 10 AU/mL	Equivocal	The equivocal sample should be retested. If the result remains in this range after repeat testing, a second sample should be collected and tested three weeks later.
≥ 10.0 AU/mL	Positive	Possible presence of detectable *Toxoplasma gondii* IgM antibodies. A specimen with a positive result should be further tested for *Toxoplasma gondii*.

Note - *The magnitude of the measured result is not indicative of the amount of antibody present.*

Limitations:

1. The test should be performed on serum only. The use of whole blood or plasma specimens has not been established.
2. The use of icteric or lipemic sera, or sera exhibiting hemolysis or microbial growth should be avoided.
3. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
4. Do not rely on any single test result as the sole determinant in diagnosing recently acquired infection. If acute infection is suspected, a patient sample should be tested for the presence of Toxoplasma – specific IgG and IgM Antibodies.
5. The performance was not evaluated in immunocompromised patients.
6. Bacterial contamination or heat inactivation of the specimens may affect the test results.

Validation Data:
Within run and between run precision was performed on both positive and negative samples for each assay, with 100% agreement.

Manufacturer's claims for assay performance are as follows:

Toxo IgG

Sensitivity: 98.8% (95% CI = 96.6-99.5%) positive agreement with comparator assay

Specificity: 94.3% (95% CI = 90.9-96.6%) negative agreement with comparator assay

Toxo IgM

Sensitivity: 98.9% (95% CI = 94.3-99.7%) positive agreement with comparator assay

Specificity: 98.9% (95% CI = 97.1-99.4%) negative agreement with comparator assay

Method comparison studies were performed using patient samples run by the improved Toxo IgG II and IgM II assays versus current Toxo IgG and IgM assays. Results are shown in tables 1 and 2 for Toxo IgG and IgM respectively. There was 100% concordance in results interpretation for both assays.

Table 1: Toxo IgG Patient Comparisons

Toxo IgG Positive and Negative Concordance			
	# Toxo IgG Positive	# Toxo IgG Negative	TOTAL
# Toxo IgG II Positive	10	0	10
# Toxo IgG II Negative	0	10	10
TOTAL	10	10	20
% Agreement	100%	100%	

Table 2: Toxo IgM Patient Comparisons

Toxo IgM Positive and Negative Concordance			
	# Toxo IgM Positive	# Toxo IgM Negative	TOTAL
# Toxo IgM II Positive	10	0	10
# Toxo IgM II Negative	0	10	10
TOTAL	10	10	20
% Agreement	100%	100%	

REFERENCES:

Diasorin LIAISON® Toxo IgM II Package Insert ([REF] 310715) December 2013

Diasorin LIAISON® Toxo IgG II Package Insert ([REF] 310705) December 2013