

# OnSite® Toxo IgG/IgM Combo Rapid Test

**REF R0234C**

## Instructions for Use

### INTENDED USE

The OnSite Toxo IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgM and IgG antibodies to *Toxoplasma gondii* (*T. gondii*) in human serum, plasma or whole blood. It is intended to be used by professionals as an aid in the diagnosis of infection with *T. gondii*.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

### SUMMARY AND EXPLANATION OF THE TEST

*T. gondii* is an obligate intracellular protozoan parasite with a worldwide distribution<sup>1,2</sup>. Serological data indicates that approximately 6-47% of the population is chronically infected with the organism<sup>3</sup>. A *T. gondii* infection occurs essentially without knowledge of the patient and may be unrelated to direct exposure to a cat (e.g., by ingestion of vegetables or water contaminated with oocysts or ingestion of undercooked meat contaminated with cysts)<sup>4</sup>. An initial *T. gondii* infection and the subsequent chronic infection are clinically undetected in 80% to 89% of healthy individual<sup>5</sup>. In immunosuppressed patients, such as patients infected with HIV, both acute and recurrent toxoplasmosis can have severe clinical manifestations<sup>5</sup>. In pregnant women, an acute *T. gondii* infection may lead to serious fetal congenital mental retardation, blindness and hydrocephaly<sup>6</sup>.

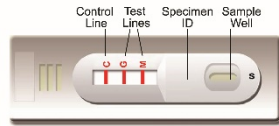
Methods for diagnosis of toxoplasmosis include PCR, histological diagnosis, parasite isolation and serology<sup>4</sup>. Serological detection of anti-*T. gondii* specific IgM and IgG antibodies is the primary method for diagnosis of toxoplasmosis. Diagnosis of toxoplasmosis is helpful in determining the risk for congenital toxoplasmosis during pregnancy<sup>4</sup>.

Anti-*T. gondii* IgM develops during acute primary infection with *T. gondii* and declines generally within a few months<sup>4</sup>. Anti-*T. gondii* IgG develops generally within 1–2 weeks post infection, peaks within 1–2 months, and usually persists for life<sup>4</sup>. The absence of IgG antibodies before or early in pregnancy allows the identification of women at risk of acquiring infection<sup>7</sup>. Additionally, the presence of IgG allows the identification of immunocompromised patients at risk for the reactivation of a latent infection<sup>7</sup>.

The OnSite Toxo IgG/IgM Combo Rapid Test detects anti-*T. gondii* IgM and IgG in human serum, plasma or whole blood by utilizing *T. gondii*-specific antigens. The test can be performed within 10 minutes by minimally skilled personnel without the use of laboratory equipment.

### TEST PRINCIPLE

The OnSite Toxo IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test strip in cassette device consists of: 1) a colored conjugate pad containing a recombinant *T. gondii* antigen conjugated with colloidal gold (Toxo conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with anti-human IgG for detection of anti-*T. gondii* IgG. The M line is pre-coated with mouse anti-human IgM for detection of anti-*T. gondii* IgM. The C line is pre-coated with a control antibody.



When an adequate volume of test specimen and sample diluent is dispensed into the sample well and buffer well, respectively, the specimen migrates by capillary action across the test strip. Anti-*T. gondii* IgM, if present in the specimen, will bind to the Toxo conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a colored M line, indicating an anti-*T. gondii* IgM positive test result. Anti-*T. gondii* IgG, if present in the specimen, will bind to the Toxo conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgG forming a colored G line, indicating an anti-*T. gondii* IgG positive test result.

Absence of any test lines (G or M) suggests a negative test result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies, regardless of color development on the test lines (G and M). If no control line (C line) develops, the test result is invalid and the specimen must be retested with another device.

### REAGENTS AND MATERIALS PROVIDED

- Individually sealed foil pouches containing:
  - One cassette device
  - One desiccant
- 10 µL capillary tubes
- Sample diluent (REF SB-R0234, 5 mL/bottle)
- Instructions for Use

### MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

- Positive control
- Negative control

### MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or timer
- Lancing device for whole blood test

### WARNINGS AND PRECAUTIONS

#### For In Vitro Diagnostic Use

- Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
- Do not open the sealed pouch until ready to conduct the assay.
- Do not use expired devices or components.
- Bring all reagents to room temperature (15-30°C) before use.
- Do not use components from another test kit to substitute for components of this kit.
- Do not use hemolyzed blood specimens for testing.
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission

of HIV, HBV and other blood-borne pathogens.

- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
- Handle negative and positive controls in the same manner as patient specimens.
- The test result should be read 10-15 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated.
- Do not perform the test in a room with strong air flow, e.g. an electric fan or strong air conditioning.

### REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

### SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

#### Plasma/Serum

- Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
- To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
- To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

#### Whole Blood

- Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

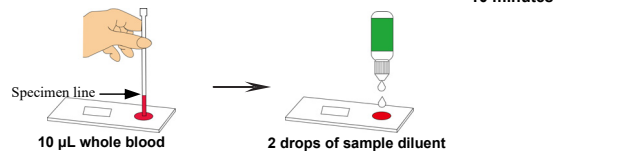
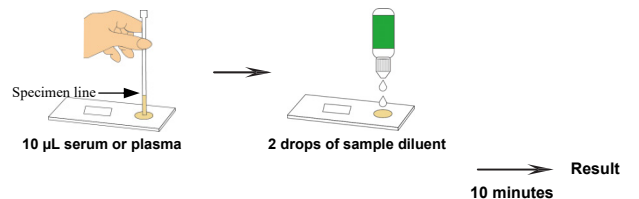
Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

### ASSAY PROCEDURE

- Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.
- When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.
- Be sure to label the device with the specimen's ID number.
- Fill the capillary tube with specimen not exceeding the specimen line as shown in the images below. The volume of specimen is approximately 10 µL. **For better precision, transfer specimen using a pipette capable of delivering a 10 µL volume.**

Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Immediately add 2 drops (about 60-80 µL) of sample diluent to the sample well with bottle positioned vertically.



- Set up the timer.

- Result should be read at 10 minutes. Positive results may be visible in as soon as 1 minute. Negative results must be confirmed at the end of 15 minutes only. **Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.**

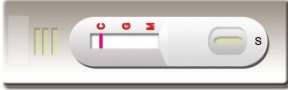
### QUALITY CONTROL

- Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.
- External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
  - A new operator uses the kit, prior to performing the testing of the specimens.
  - A new lot of test kits is used.
  - A new shipment of test kits is used.
  - The temperature during storage of the kits falls outside of 2-30°C.

- e. The temperature of the test area falls outside of 15-30°C.
- f. To verify a higher than expected frequency of positive or negative results.
- g. To investigate the cause of repeated invalid results.

**INTERPRETATION OF ASSAY RESULT**

1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that anti-*T. gondii* antibodies are not detected in the specimen. The result is negative or non-reactive.



2. **POSITIVE RESULT:**  
 2.1 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-*T. gondii* IgM. The result is anti-*T. gondii* IgM positive or reactive and anti-*T. gondii* IgG negative or non-reactive.



- 2.2 In addition to the presence of the C line, if only the G line develops, the test indicates the presence of anti-*T. gondii* IgG. The result is anti-*T. gondii* IgG positive or reactive and anti-*T. gondii* IgM negative or non-reactive.



- 2.3 In addition to the presence of the C line, if both the M and the G lines develop, the test indicates the presence of both anti-*T. gondii* IgM and IgG. The result is anti-*T. gondii* IgM and IgG positive or reactive.



*Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.*

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color development on the test lines (M and G) as indicated below. Repeat the assay with a new device.



**PERFORMANCE CHARACTERISTICS**

1. **Analytical Sensitivity of IgG Detection**  
 Twenty groups of matrix were spiked with the WHO International Standard Anti-Toxoplasma Serum Ig (TOXM) concentrations of 0.625, 1.25, 2.5, 5, and 10 IU/mL. The specimens were run on the OnSite Toxo IgG/IgM Combo Rapid Test. Defined as the 95% detection level, the limit of detection or sensitivity for the OnSite Toxo IgG/IgM Combo Rapid Test IgG test line is 2.5 IU/mL.

IgG (IU/mL)	0.625	1.25	2.5	5	10
Number Positive	0	11	19	20	20
Number Negative	20	9	1	0	0

2. **Accuracy of IgG Detection**  
 A total of 237 clinical specimens were collected and tested on the OnSite Toxo IgG/IgM Combo Rapid Test and by commercial ELISA. Comparison for all subjects showed 94.9% overall agreement for the IgG test line.

3. **Accuracy of IgM Detection**  
 A total of 231 clinical specimens were collected and tested on the OnSite Toxo IgG/IgM Combo Rapid Test and by commercial ELISA. Comparison for all subjects showed 97.8% overall agreement for the IgM test line.

4. **Performance on BBI Anti-*T. gondii* Mixed Titer Performance Panel**  
 The performance of the OnSite Toxo IgG/IgM Combo Rapid Test was evaluated using the BBI (Boston Biomedical Inc) Anti-*T. gondii* Mixed Titer Performance Panel (PTT202) and compared with three commercial immunoassays. The comparison is shown in the following table:

BBI Reference Panel (PTT202)	Abbott ARCHITECT	bioMerieux VIDAS	Diasorin LIAISON	OnSite Toxo IgG/IgM Combo Rapid Test
IgG Positive	20	20	20	20
IgG Negative	1	1	1	1
IgM Positive	8	7	6	6
IgM Negative	13	14	15	15

5. **Cross-Reactivity**  
 No false positive anti-*T. gondii* IgG and IgM results were observed on 3-14 specimens from the following disease states or special conditions, respectively:

CMV	Dengue	HAV	HBV	HCV
HIV	HSV-1	HSV-2	hCG	<i>H. pylori</i>
Malaria	TB	<i>T. pallidum</i>	Rubella	ANA
HAMA	RF (up to 8400 IU/mL)			

6. **Interference**  
 Common substances (such as pain and fever medication and blood components) may affect the performance of the OnSite Toxo IgG/IgM Combo Rapid Test. This was studied by spiking these substances into IgM positive, medium-level IgG positive, weak-level IgG positive, and IgM and

IgG negative specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the OnSite Toxo IgG/IgM Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Salicylic acid	4.24 mmol/L
4. EDTA	3.4 µmol/L	9. Sodium citrate	3.8%
5. Glucose	55 mmol/L		

**LIMITATIONS OF TEST**

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of IgG and IgM antibodies to *T. gondii* in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
2. The OnSite Toxo IgG/IgM Combo Rapid Test is limited to the qualitative detection of antibodies to *T. gondii* in serum, plasma or whole blood. The intensities of the test lines do not have linear correlation with the antibody titers in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-*T. gondii* antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with *T. gondii*.
4. A negative or non-reactive result can occur if the quantity of the anti-*T. gondii* IgG or IgM present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of the disease in which a sample is collected.
5. The OnSite Toxo IgG/IgM Combo Rapid Test has not been validated on specimens from neonates.
6. Infection may progress rapidly. If the symptom persists, while the result from OnSite Toxo IgG/IgM Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
7. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

**REFERENCES**

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7. Calderaro A, Piccolo G, Peruzzi S, et al. Evaluation of *Toxoplasma gondii* Immunoglobulin G (IgG) and IgM assays incorporating the new Vidia analyzer system. *Clin Vaccine Immunol* 2008. 15(7):1076-9.

**Index of Symbols**

	Consult instructions for use		For <i>in vitro</i> diagnostic use only		Use by
	Catalog #		Lot Number		Tests per kit
	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		



13855 Stowe Drive  
 Poway, CA 92064, USA  
 Tel: 858-457-8698  
 Fax: 858-535-1739  
 E-mail: info@ctkbiotech.com

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