

**Toxoplasma IgM  
Capture Enzyme  
Immunoassay**

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

**Method:** Diamedix Corp., Immun simplicity®

Manual or in conjunction with one of the Diamedix Automated EIA Systems such as the MAGO Plus, the DSX, or the DS2. For *In Vitro* Diagnostic Use.

**Clinical Significance**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that is distributed worldwide. *Toxoplasma gondii* exists in three forms: trophozoites, cysts and oocysts (1). The trophozoite is the invasive form present during the acute phase of infection. Tissue cysts are formed after multiplication of the organism within the host cell cytoplasm which may contain up to several thousand organisms. Oocysts develop in the intestinal epithelial cells of cats and are not found in other hosts. Oocysts are excreted in the feces of cats and mature after a few days. Humans can acquire the infection in many ways, either by accidental ingestion of oocysts shed in cat feces, by the ingestion of rare or raw meats, *in utero*, or by transfusion. Following primary infection, the parasites multiply locally and are then transported to other organs forming tissue cysts which persist for the life of the host(1,2).

Most infections (80 to 90%) are benign with few or no symptoms. Severe symptoms are seen, however, with congenital infections or those in compromised patients (3). The risk and severity of fetal infection vary according to the trimester of pregnancy in which the mother becomes infected. Women infected during their first trimester are less likely to pass the infection to the fetus; but if transmission occurs, severe outcomes such as spontaneous abortion and hydrocephalus are more likely. Disease acquired later in pregnancy, when transmission to the fetus occurs more often, tends to cause less severe, but nonetheless serious congenital manifestations such as cerebral calcifications and learning disabilities (4). Compromised host infections can lead to severe complications with significant central nervous system involvement. In AIDS infected individuals previously infected with *T. gondii*, toxoplasmic encephalitis due to reactivation of the protozoan is a major cause of morbidity and mortality (5).

The diagnosis of toxoplasmosis is frequently dependent upon serological data since the signs and symptoms of this disease often mimic those of other diseases and since isolation of *T. gondii* from the patient is difficult and unreliable. Specific antibodies can be detected during the acute phase of infection. IgM antibodies may appear as early as 5 days after infection, rise sharply, and fall to low or undetectable levels within weeks or months in the majority of patients. IgG antibodies generally appear 1-2 weeks after the infection, reach a peak in 6 to 10 weeks and persist at various levels for the rest of the life of the host (4,6,7,8).

The traditional methods of detecting IgM antibodies to *Toxoplasma gondii*, such as the indirect fluorescent antibody test, have now been replaced in many settings by enzyme immunoassays (EIAs) which are less cumbersome to perform and more amenable to automation. Capture EIAs offer the additional advantage of avoiding interference due to rheumatoid factor and competing IgG antibodies.

The Diamedix Immunosimplicity® (Is)-*Toxoplasma* IgM Capture Test Kit is a Capture EIA procedure intended for the presumptive qualitative detection of IgM antibodies to *Toxoplasma gondii* antigen. The test can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems.

## **Principle of the Procedure**

The Is-*Toxoplasma* IgM Capture Test Kit utilizes ELISA based on the antibody-capture technique. Diluted patient sera are incubated with mouse monoclonal antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. Patient anti-*Toxoplasma* IgM antibodies are 'detected' and bound by an immunocomplex, Enzyme Tracer, consisting of toxoplasma antigen which is linked to a mouse monoclonal anti-toxoplasma antibody conjugated to horseradish peroxidase. Unbound tracer is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgM antibodies to *Toxoplasma gondii* antigen present in the sample.

## **Specimen Collection**

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2 to 8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples are to be frozen at -20°C. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens

containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used. The NCCLS provides recommendations for collecting and storing blood specimens (9).

**CAUTION:** Serum samples must not be heat-inactivated prior to use.

## Reagents

<b>Anti-IgM Coated Wells</b>	Twelve, 8-well microwell breakapart strips, color-coded yellow, coated with mouse monoclonal anti-human IgM (heavy chain).
<b>Cut-Off Calibrator</b>	One vial with blue cap containing 0.25 ml of human serum, preserved with 0.1% sodium azide, weakly reactive for <i>Toxoplasma gondii</i> IgM antibodies. The Cut-Off Calibrator is used to determine the cut-off of the assay.
<b>Low Positive Control</b>	One vial with white cap containing 0.25 ml of human serum preserved with 0.1% sodium azide. Assigned range printed on label. The Positive Control is used to control the low range of the assay.
<b>Negative Control</b>	One vial with black cap containing 0.25 ml of human serum preserved with 0.1% sodium azide, non-reactive for <i>Toxoplasma gondii</i> IgM antibodies. The Negative Control is used to control the negative range of the assay.

*Note: The Cut-Off Calibrator and Controls are prepared from different serum lots.*

<b>Lyophilized Antigen</b>	Six vials of lyophilized <i>Toxoplasma gondii</i> antigen, whole cell sonicate, strain RH.
<b>30X Tracer</b>	One vial with red cap containing 0.8 ml mouse monoclonal anti- <i>Toxoplasma gondii</i> conjugated to horseradish peroxidase (30X concentrate) in stabilizer.
<b>Tracer Diluent</b>	One bottle with red cap containing 25 ml borate buffer. Also includes protein stabilizers, gentamycin and Proclin™ 300 as preservatives. Color-coded pink.
<b>Sample A Diluent</b>	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Color-coded blue.

- Wash S Concentrate** Two bottles with clear caps containing 50 ml of Phosphate buffered saline with Proclin™ 300, 15 ppm active ingredient. Color-coded light blue/green. Each bottle is sufficient to make 1 liter of wash solution.
- Substrate G** One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5'-tetramethylbenzidine).
- Stop M Solution** One bottle with white cap containing 30 ml of 1 N Phosphoric and 1N Hydrochloric acids. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

These reagents should be stored at 2 to 8° C.

## Other Materials Required

### Manual Users:

1. Wash bottle or automated microplate washer
2. Pipettors capable of dispensing appropriate volumes
3. Timer
4. One liter graduated cylinder
5. One liter wash solution reservoir
6. Deionized or distilled water
7. Absorbent toweling
8. Tubes or microwell plate for serum dilution
9. Reader capable of reading absorbance at 450nm, reference at 600-630 nm.
10. Incubator capable of maintaining temperature of  $37 \pm 3^{\circ}\text{C}$

### Diamedix Automated EIA System Users:

1. One liter graduated container
2. Deionized or distilled water
3. Dilution containers as appropriate to system
4. Sample and Reagent tips required by system
5. Reagent containers required by system

### Warnings:

1. Handle samples, Cut-Off Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the Cut-Off Calibrator and controls has been found negative for Hepatitis B surface antigen, HCV and HIV-I and 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 and 2, Hepatitis B virus or Hepatitis C virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National

Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.

2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin™ 300 as a preservative. When disposing of reagents containing Proclin™ 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Serum components contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.
6. Sodium azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into the immunocomplex and substrate steps.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. The substrate contains 3,3' 5,5' Tetramethylbenzidine (TMB) which has shown possible mutagenic effects in laboratory experiments.

## Calibration

This test uses an in-house reference standard (Cut-Off Calibrator). The Cut-Off Calibrator has been derived from weakly positive sera and is titrated to an absorbance value equivalent to the cut-off of the assay. Samples whose absorbances exceed this value are considered positive for Toxoplasma IgM antibodies and samples whose absorbances are less than this value are considered negative for Toxoplasma IgM antibodies. To account for the inherent variations in enzyme immunoassays an equivocal range of  $\pm 10\%$  has been included at the assay cut-off.

## Quality Control

- a) The Low Positive and Negative Controls must be included in each test run.
- b) The absorbance of the Blank must be  $< 0.100$ .
- c) The absorbance of the Cut-Off Calibrator must be  $\geq 0.150$  when read against the blank.
- d) The Low Positive and Negative Controls must be within their assigned ranges.

**If any of these criteria is not met, the run is invalid and must be repeated.**

**NOTES:** Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. For guidance on appropriate Quality Control practices, please refer to NCCLS document C24-A, Internal Quality Control Testing: Principles and Definitions.

## **Procedure**

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate(20X) to one liter of deionized or distilled H<sub>2</sub>O.

Each vial of lyophilized antigen is sufficient for at least 2 strips. Reconstitute only the number of vials required. Discard any unused Enzyme Tracer after the day's testing is completed.

### **MANUAL USERS:**

1. Prepare 1:101 dilutions of the Cut-Off Calibrator (in triplicate), controls and patient samples in Sample Diluent. (e.g., by addition of 2  $\mu$ l sample to 200  $\mu$ l Sample Diluent or 5  $\mu$ l sample to 500  $\mu$ l Sample Diluent)
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100  $\mu$ l of diluted Calibrator, controls and patient samples, to the wells. Avoid formation of bubbles when transferring diluted samples.  
*Note: Include one well which contains 100  $\mu$ l of Sample Diluent as a reagent blank. This will ultimately be used to "zero" the photometer before reading test results. DO NOT ADD ENZYME TRACER TO THE BLANK WELL.*
3. Allow the wells to incubate uncovered at  $37 \pm 3^{\circ}\text{C}$  for  $60 \pm 5$  minutes.
4. As soon as the sample incubation has commenced, prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent to each vial of lyophilized antigen required for the run. Mix until all the lyophilized material is reconstituted. Then add 100  $\mu$ l 30X Tracer to each antigen vial and mix well. Allow the Enzyme Tracer to sit at room temperature ( $18\text{-}30^{\circ}\text{C}$ ) for at least 30 minutes.
5. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300  $\mu$ l each of Wash Solution. After the 3<sup>rd</sup> volume of Wash Solution, allow the wells to "soak" for at least one minute prior to final aspiration/emptying. When using an automated washer, follow the manufacturer's instructions and set up the same wash procedure as described.
6. Place 100  $\mu$ l of Enzyme Tracer into each well (except the blank), avoiding bubble formation.

7. Add 100 µl Sample Diluent to the Blank well.
8. Allow the wells to incubate uncovered at 37 ± 3°C for 60 ± 5 minutes.
9. Wash the wells as described in Step 5 above.
10. Place 100 µl of Substrate into each well, avoiding bubble formation.
11. Allow the wells to incubate uncovered at 37 ± 3°C for 20 ± 2 minutes.
12. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
13. Read the absorbance of the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 minutes of adding Stop Solution.

**Diamedix Automated EIA System Users:**

If using one of Diamedix's Automated EIA Systems, please refer to the corresponding Operating Manual for the test setup, procedure, and accessories/consumables needed.

**Calculation of Results**

Calculate the MEAN absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance exclude any absorbance value that deviates by more than 20% from the mean absorbance value. Calculate the mean absorbance value from the two remaining absorbances. Exclusion of more than one of the 3 absorbance values invalidates the run. Determine the Index Value for each patient sample or control using the following formula:

$$\frac{\text{Absorbance of Sample}}{\text{Mean Absorbance of Cut-Off Calibrator}} = \text{Index Value}$$

*Example: Absorbance values obtained for Cut-Off Calibrator: 0.456, 0.445, 0.458 (after subtraction of the Blank)  
 Mean Absorbance of the Cut-Off Calibrator = 0.453  
 Sample Absorbance = 0.959  
 Index Values are then calculated as follows:  
 Sample Absorbance / Mean Absorbance of Cut-Off Calibrator = 2.13*

When using Diamedix Automated EIA Systems, results are automatically calculated and expressed as Positive, Equivocal or Negative.

**Reference Ranges**

**CAUTION: Results must be interpreted in conjunction with toxoplasma IgG results. Please refer to "General Guidelines for Interpretation of Toxoplasma gondii Serology results" as found in Section 5. of Interpretation of Results in the package insert.**

The following is only a guide to interpretation. **Each laboratory can establish its own "normal" ranges based on populations encountered.**

<u>Index Value</u>	<u>Interpretation</u>
Index < 0.90	Negative for anti-Toxoplasma IgM.
Index $\geq$ 1.10	Positive for anti-Toxoplasma IgM.
Index 0.90-1.09	Equivocal for anti-Toxoplasma IgM.*

Note that the magnitude of the Index Value is not indicative of the total amount of antibody present.

\* When equivocal results are obtained, another specimen should be collected ten to fourteen days later and tested in parallel with the initial specimen. If the second sample is also equivocal, antibody status cannot be determined. Other clinical and serological evidence should be sought in those cases. If the second sample is presumptive positive, seroconversion has occurred and may be indicative of a current or recent infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question, is defined as seroconversion, and indicates primary or recent infection.

A negative result does not always exclude the possibility of *Toxoplasma gondii* infection. The sample may have been collected before appearance of IgM antibody. If infection is suspected, a second sample should be collected at 7-14 days later and tested concurrently with the first sample to determine if seroconversion has occurred.

## Reporting Results

When reporting results the following statement should be included: "The following results were obtained with the Diamedix Immunosimplicity Is-Toxoplasma IgM Capture EIA Test Kit. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present."

## Procedure Notes

1. The concentrations of anti-Toxoplasma IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
2. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
3. Do not use reagents beyond their expiration date.
4. Store unused reagents at 2 to 8° C.
5. Incubations above or below the recommended temperatures or times may give erroneous results.

6. The Capture ELISA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
7. Coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
8. (*Manual Procedure Only*) The washing procedure is very important and requires special attention. (Please refer to the Procedure section)  
**NOTE:** *Improperly washed wells may give erroneous results.*

## **Limitations**

1. The results obtained with the Is-Toxoplasma IgM Capture Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pre-test likelihood of Toxoplasmosis being present. Testing should only be done when clinical evidence suggests the diagnosis of Toxoplasmosis.
3. This test is not intended for the determination of immune status. It is intended for the determination of a patient's antibody response to indicate active infection to *Toxoplasma gondii* and not as an indication of immunity.
4. Assay performance characteristics have not been established for visual result determination.
5. Assay performance characteristics for the use of specimen matrices other than serum have not been established.
6. Assay performance characteristics have not been established with single wavelength spectrophotometers.
7. Specific IgM antibodies are usually detected in patients with recent primary infection, but they may be found in patients with reactivated or secondary infections, and they are sometimes found in patients with no other detectable evidence of recent infection.
8. Low levels of IgM antibodies may occasionally persist for more than 12 months post-infection. Such a residual antibody response may be distinguished from the early IgM response to active infection by testing sera from the patient 2-4 weeks later using the Is-Toxoplasma IgM Capture Test Kit and by reference to changing levels of Toxoplasma IgG antibodies (Diamedix Catalog #720-300).
9. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
10. Performance characteristics have not been established for newborns, for cord blood or for immunosuppressed individuals (including HIV-positive and pre-and post-transplant patients).

11. Performance characteristics of the Diamedix Is-Toxoplasma IgM Capture Test Kit with automated equipment other than Diamedix Automated EIA Systems have not been established.

## References

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9. Procedures for the Handling and Processing of Blood Specimens: Approved Guideline-Second Edition NCCLS Document H18-A2, Vol. 19, No. 21.1999.

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