

## **CLSI DISCLAIMER:**

The Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), Procedure Guides are provided as a courtesy for you to use in preparing your own laboratory's CLSI Procedure Manuals. Diamedix is not responsible for any modification made by the user, to these documents.

For Individual Laboratory to Complete:

**Toxoplasma IgG  
Enzyme Immunassay**

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

**Method: Diamedix Corp., Immunosimplicity®**

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 oocytes (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. Oocytes develop in the intestinal epithelial cells of cats and are not found in other hosts. Oocytes 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 oocytes 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 antibodies to *Toxoplasma gondii*, such as the Sabin-Feldman dye test, the indirect hemagglutination test and 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.

The Diamedix Immunosimplicity<sup>®</sup> Is-Toxoplasma IgG Test Kit is an EIA procedure intended for the qualitative and quantitative detection of antibodies to *Toxoplasma gondii* antigen. The test can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems. The results are reported in IU/ml, which are traceable to the WHO 3rd International Standard for Anti-Toxoplasma Serum, 1994 (9).

### **Principle of the Procedure**

Diluted samples are incubated with *Toxoplasma gondii* antigen bound to the solid surface of a microtiter well. If IgG antibodies against *Toxoplasma gondii* are present in the samples they will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgG) is added and will bind to these complexes. Unbound conjugate 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 IgG 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.

For the diagnosis of recent *Toxoplasma* infection, paired sera should be obtained at least three weeks apart and tested in the same run.

<b>CAUTION:</b> Serum samples must not be heat-inactivated prior to use.
--

**Reagents****Antigen Wells**

Twelve, 8-well microwell breakapart strips, color-coded silver, coated with partially purified *Toxoplasma gondii* antigen (Deelen strain produced in HEP-2 cell line (10), detergent extract).

**0 IU/ml Standard**

One vial with yellow cap containing 1.8 ml of pre-diluted human serum, non-reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned IU/ml value printed on label.

**50 IU/ml Standard**

One vial with green cap containing 1.8 ml of pre-diluted human serum, weakly reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned IU/ml value printed on label.

**250 IU/ml Standard**

One vial with red cap containing 1.8 ml of pre-diluted human serum, moderately reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90ppm active ingredient. Assigned IU/ml value printed on label.

**High Positive Control**

One vial with white cap containing 1.8 ml of pre-diluted human serum, highly reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned IU/ml range printed on label.

**Low Positive Control**

One vial with blue cap containing 1.8 ml of pre-diluted human serum, weakly reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned IU/ml range printed on label.

**Negative Control**

One vial with black cap containing 1.8 ml of pre-diluted human serum, non-reactive for *Toxoplasma gondii* IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient.

*Note: Standards and Controls are prepared from different serum lots.*

**Sample A Diluent**

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.

<b>Conjugate</b>	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
<b>Substrate HRP</b>	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
<b>Stop M Solution</b>	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1N Hydrochloric acids. <b>CAUTION:</b> Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

<p><b>These reagents should be stored at 2 to 8° C.</b></p>
---

### 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, Standards, controls and the materials that contact them as potential biohazards. Each donor unit in the Standards 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, 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 conjugate activity. Clean pipet tips MUST be used for conjugate addition so that azide is not carried over from other reagents.
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 been shown to cause possible mutagenic effects in laboratory experiments.

### **Calibration**

This test uses a 3-point calibration system (with qualitative reporting optional) based on reference standards. These standards have been prepared from serum that is strongly positive for the antibody under investigation. The Standards have been assigned unitages in International units(IU)per ml, traceable to the WHO 3rd International Standard for anti-Toxoplasma Serum,1994 (9).

The positive cut-off has been assigned a value of 50 IU/ml and a Standard prepared at that level. The upper end of the reportable range has been assigned a value of 250 IU/ml and a Standard prepared at this level. The 0 IU/ml Standard is prepared from material devoid of the antibody in question. The test can be performed using all three Standards and reading the results from the point to point standard curve produced. If the user desires a qualitative result, i.e. negative or positive, then only the 50 IU/ml Standard needs to be tested and patient absorbance values obtained compared to the 50 IU/ml Standard absorbance.

Patient samples which contain high levels of antibody may exceed the absorbance of the highest Standard. Such patient sample results should be reported as "Greater than 250 IU/ml". If numerical results are required for such samples, pre-dilute the sample using Sample Diluent and re-assay. The resulting IU/ml value should then be multiplied by the dilution factor to obtain estimated values.

### **Quality Control**

- a) The High Positive, Low Positive and Negative Controls must be included in each test run.

- b) The absorbance of the Blank or the 0 IU/ml Standard must be < 0.2.
- c) The absorbance of the 50 IU/ml Standard must be higher than that of the Negative Control.
- d) The absorbance of the 50 IU/ml Standard must be lower than that of the Low Positive Control.
- e) The absorbance of the 250 IU/ml Standard must be higher than that of the Low Positive Control.
- f) The Low Positive Control must be within its assigned range.
- g) The High Positive Control must be >250 IU/ml.
- h) The Negative Control must be < 40 IU/ml.

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

**Notes:** The Negative and Positive Controls are intended to monitor substantial reagent failure. The controls will not control all parts of the procedure such as technical dilution of patient specimens. The Positive Controls will not ensure precision at the assay cut-off. Users may wish to establish an in-house control having a quantitative value determined by replicate testing, at or near the cut-off to monitor the precision of the assay cut-off. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

### **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.

#### **Manual Users:**

**The Standards and Controls are provided ready to use: DO NOT DILUTE FURTHER.**

**Note:** *For qualitative assays,* the 50 IU/ml Standard only is required. This Standard should be assayed in triplicate. In addition, a Blank (100 µl Sample Diluent only, in the first well of the first strip) is required and will be used to "zero" the photometer before reading test results.

*For quantitative assays,* all three Standards are required. No Blank is required; the 0 IU/ml Standard will function as the 'zero' and will be placed in the first well of the first strip. Standards can be run singly or in duplicate.

**High Positive, Low Positive and Negative Controls must be run for either assay option.**

1. Prepare 1:101 dilutions of the patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl 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 µl of Standards, controls and diluted patient sample, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
3. Allow the wells to incubate uncovered at  $37 \pm 3^{\circ} \text{C}$  for  $60 \pm 5$  minutes.
4. 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 µl of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at  $37 \pm 3^{\circ} \text{C}$  for  $60 \pm 5$  minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at  $37 \pm 3^{\circ} \text{C}$  for  $20 \pm 2$  minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

Refer to the BP-96 Plate Reader Operation Manual for complete instructions on set-up and operating procedures.

**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**

**Qualitative Assay:** Qualitative results may be obtained using the 50 IU/ml Standard only in triplicate, following a single Blank well (100 µl Sample Diluent only). If performing the qualitative assay option, manually set the reader for absorbance mode or cut-off control test mode and calculate the mean absorbance for the three Standard wells.

**Note:** When calculating the mean absorbance exclude any absorbance value that deviates by more than 15% 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.

*Example: Absorbance values obtained for 50 IU/ml Standard: 0.456, 0.445, 0.458 (after subtraction of the Blank)*  
*Mean Absorbance of the 50 IU/ml Standard = 0.453*  
*Sample Absorbance = 0.959*  
*Index Values are then calculated as follows:*  
*Sample Absorbance / Mean Absorbance of 50 IU/ml Standard = 2.13*

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

**Quantitative Assay :** Quantitative results may be obtained from the point-to-point curve fit using all three Standards. For plate readers, the point-to-point option should be selected and Standard values entered accordingly. Index Values can be calculated by dividing the IU/ml values by 50 (the positive cut-off value).

The Diamedix Automated EIA Systems will calculate results using the point-to-point curve fit and will print results automatically.

### Reference Ranges

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

Less than 40.0 IU/ml Index < 0.80	Negative for anti-Toxoplasma IgG.
Greater than/equal to 50.0 IU/ml Index $\geq$ 1.0	Positive for anti-Toxoplasma IgG.
40.0 to 49.9 IU/ml Index 0.8-0.99	Equivocal for anti-Toxoplasma IgG.*

Note that when using the assay qualitatively the magnitude of the Index Value has no relevance and results should be reported as under "Interpretation" above.

\* 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, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample is positive, the patient can be considered to have a primary 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 infection. The sample may have been collected before appearance of IgG antibody. If infection is suspected, a second sample should be collected at least 3 weeks later and tested concurrently with the first sample to determine if seroconversion has occurred.

### Reporting Results

When the IU/ml value is reported for a single specimen the following statement should be included : "The following results were obtained with the Diamedix Immunosimplicity Is-Toxoplasma EIA Test System. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgG level cannot be correlated to an end-point titer."

When reporting semi-quantitatively results a 3.8-fold or greater difference between acute and convalescent specimen IU/ml values indicates a significant increase in antibody level.

When the assay is used semi-quantitatively, the following statement should be included when reporting results: "Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall again to lower or undetectable levels within a month. Other patients may not develop significant antibody levels. Culture results, serology and antigen detection methods should all be appropriately used along with clinical findings for diagnosis".

### **Paired Sera**

To determine a significant difference between acute/convalescent sera, both specimens should be run within the same assay. Paired sera should be evaluated within the linear range of the assay. Studies performed both manually and using Diamedix Automated EIA Systems have shown that, overall, a 3.8-fold or greater increase in the IU/ml ratio (convalescent serum IU/ml value/ acute serum IU/ml value) corresponds to a four-fold increase in Toxoplasma IgG antibody level and a 2.2-fold increase in the IU/ml ratio corresponds to a two-fold increase in Toxoplasma IgG antibody level. Ratios in the range of 2.2 to 3.8 may be considered equivocal for significant increase status. In this case, paired samples can be retested or additional samples collected if necessary. If paired sera controls are desired, it is recommended that a 1:4 dilution of a sample with an IU/ml value of between 200 and 250 be prepared in Sample Diluent. The undilute and 1:4 diluted material will provide a simulated serum pair. The Ratio of the undilute and 1:4 diluted material can then be compared against the established range.

### **Procedure Notes**

1. The concentrations of anti-Toxoplasma IgG 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 **HRP** 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 EIA 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. Antigen 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 IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Assay performance characteristics have not been established for visual result determination.
3. Assay performance characteristics for the use of specimen matrices other than serum have not been established.
4. A single positive result only indicates previous immunologic exposure; the level of antibody response or class of antibody may not be used to determine active infection or disease stage. A test for IgM antibodies should be performed for individuals suspected of primary infection with *Toxoplasma gondii*.
5. Assay performance characteristics have not been established with single wavelength spectrophotometers.
6. Performance characteristics have not been established for newborns, using cord blood or for immunosuppressed individuals (including HIV-positive and pre-and post-transplant patients).
7. Performance characteristics of the Diamedix Is-Toxoplasma IgG Test Kit with automated equipment other than Diamedix Automated EIA Systems have not been established.
8. The assay's lower and upper linearity limits are 50 to 250 IU/ml.

### **References**

1. Remington, J.S. 1973. Toxoplasmosis. In : Obstetrics and Perinatal Infections. Charles, D. and Finland, M. (eds). Lea & Febiger, p.27-74.
2. Krick, J.A. and Remington, J.S. 1978. Toxoplasmosis in the Adult- An Overview. N. Engl. J. Med. 298 No. 10: 550-553.
3. Guerina, N. G. 1994. Congenital Infection with *Toxoplasma gondii*. Pediatric Annals. 23:3:138-151.
4. Bryan, R.T. and Wilson, M. 1988. Toxoplasmosis. Lab. Management. 26: 40-43.
5. Luft. B.J. and Remington, J. S. 1992. Toxoplasmic Encephalitis in AIDS. Clin. Infect. Dis. 15: 211-222.
6. Palmer, D.F., Walls, K., Cavallaro, J.J. and Wilson, M. 1976. Serology of Toxoplasmosis. U.S. Dept. of Health, Education and Welfare, PHS, CDC, Atlanta, GA.
7. Walls, K.W. 1978. Serodiagnosis of Toxoplasmosis. Lab. Management. Jan. 27-31.

8. Turgeon, M. L. 1996. Toxoplasmosis. In: Immunology and Serology in Laboratory Medicine. 2nd Edition. Mosby. p. 287-294.
9. The International Standard for Anti-Toxoplasma Serum, human (3rd International Standard Preparation). WHO International Laboratory for Biological Standards and NIBSC. 1994.
10. Van Loon A.M. and Van Der Veen, J. 1980. Enzyme-linked Immunosorbent Assay for Quantitation of Toxoplasma Antibodies in Human Sera. J.Clin. Pathol. 33: 635-639.

Proclin<sup>™</sup> 300 is a trademark of Rohm and Haas Corp. Philadelphia, PA.