Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

Toxoplasma IgG
Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-300 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of patient samples in Sample Diluent. Mix well.
   Note that Standards and Controls are ready-to-use.
2. Add 100 µl of Standards, Controls and diluted patient samples into the antigen wells.
3. Incubate at 37 ± 3° C for 60 ± 5 min.
4. Discard contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at 37 ± 3° C for 60 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at 37 ± 3° C for 20 ± 2 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the qualitative, semi-quantitative and quantitative detection of IgG antibodies to *Toxoplasma gondii* in human serum by indirect enzyme immunoassay to aid in the assessment of the patient's immunological response to infection with *Toxoplasma gondii* and in the determination of the immune status of individuals, including females of child-bearing age. The evaluation of acute and convalescent sera can aid in the diagnosis of primary or reactivated infection with *Toxoplasma gondii*. This product is not FDA cleared for use in screening blood and plasma donors.

SUMMARY AND EXPLANATION

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® *Is*-Toxoplasma IgG Test Kit is an EIA procedure intended for the qualitative and quantitative detection of antibodies to *Toxoplasma gondii* antigen. 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.

REAGENTS

Each *Is*-Toxoplasma IgG Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded silver, coated with partially purified *Toxoplasma gondii* antigen (RH strain produced in HeLa cell line).
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	Two vials 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, 90 ppm 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 that 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 (20X)	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 1050 ml of wash solution.
Conjugate	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.
Substrate **HRP**	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.

Store these reagents at 2 to 8° C.

Other Materials Required

Manual Users:

Wash bottle or automated microplate washer.

Pipettors capable of dispensing appropriate volumes.

Timer.

One liter graduated cylinder.

One liter wash solution reservoir.

Deionized or distilled water.

Absorbent toweling.

Tubes or microwell plate for serum dilution.

Reader capable of reading absorbance at 450 nm, reference at 600-630 nm.

Incubator capable of maintaining temperature of 37 ± 3°C

Automated EIA Processor Users:

One liter graduated cylinder.

Deionized or distilled water.

Pre-dilution cups, strips or plates.

ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

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-1 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.[11]
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. Reagents containing Sodium Azide:
   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

   European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.

   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
6. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.

ADDITIONAL PRECAUTIONS

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. Expiration dates are printed on the reagent labels.
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.*

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques.[13] 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 (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should 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 then tested in the same run.

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

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_2O.

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 samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
3. Allow the wells to incubate uncovered at 37 ± 3° C for 60 ± 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 ± 3° C for 60 ± 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 +_3° C for 20 +_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.

Automated EIA Processor Users:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

QUALITY CONTROL

1. The High Positive, Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank or the 0 IU/ml Standard must be < 0.2.
3. The absorbance of the 50 IU/ml Standard must be higher than that of the Negative Control.
4. The absorbance of the 50 IU/ml Standard must be lower than that of the Low Positive Control.
5. The absorbance of the 250 IU/ml Standard must be higher than that of the Low Positive Control.
6. The Low Positive Control must be within its assigned range.
7. The High Positive Control must be > 250 IU/ml.
8. The Negative Control must be < 40 IU/ml.

If any of these criteria are not met, the results are invalid and the test should 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. For guidance on appropriate quality control practices, please refer to CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

***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 an Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor), 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).

An Automated EIA Processor will automatically calculate results using the point-to-point curve fit and will then print results.

Specimens which yield absorbances greater than that of the 250 IU/ml Standard may be reported as greater than 250 IU/ml or Index >5.0. Alternatively, such samples may be pre-diluted in Sample Diluent and retested. The resulting IU/ml or Index Value must be multiplied by the dilution factor for reporting.

Example: If the specimen has been pre-diluted 1:5 before testing, the resulting IU/ml or Index Value should be multiplied by 5.

2. Interpretation

IU/ml	Index Value	Interpretation
< 40.0	< 0.80	Negative for anti-Toxoplasma IgG
40.0 – 49.9	0.8 – 0.99	Equivocal for anti-Toxoplasma IgG*
>_50.0	>_1.0	Positive for anti-Toxoplasma IgG

Note that when using the assay qualitatively the magnitude of the Index Value has no significance 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 (see Section 4. Paired Sera). 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 active 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.

3. 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 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-quantitative results a 3.8-fold or greater difference between acute and convalescent specimen IU/ml values indicates a significant increase in antibody level (see 4. Paired Sera, below). 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".

4. Paired Sera

To determine a significant difference between acute/convalescent sera, both specimens must be run within the same assay. Paired sera should be evaluated within the linear range of the assay In-house studies performed both manually and using the MAGO Plus 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.

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-Toxoplasma IgG Test Kit cut-off value has been set at 50 IU/ml based on the WHO 3rd International Standard for Anti-Toxoplasma Serum, Human. This cut-off was established to optimally differentiate those individuals with, from those without, immunological experience to *Toxoplasma gondii.*

This cut-off value was verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to two hundred and sixty-seven normal sera assayed manually by Diamedix in the *Is*-Toxoplasma IgG Test Kit and another commercially available test method. Twenty five of these

samples were a commercially obtained performance panel with accompanying results from a variety of commercially available procedures. At the optimized cut-off level, the Diamedix *Is*-Toxoplasma IgG Test Kit has a relative sensitivity of 90% and a relative specificity of 99% based on comparison to the marketed test.

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 the MAGO® Plus Automated EIA Processor have not been established.
8. The assay's lower and upper linearity limits are 50 to 250 IU/ml.

EXPECTED VALUES

The prevalence of Toxoplasma IgG antibodies in the normal population can vary depending on a number of factors such as age, geographical location, socioeconomic status, race and type of test used. It has been estimated that in the United States 8-20% of the normal population has anti-toxoplasma IgG antibodies.[1] For females of child-bearing age and for pregnant females prevalence rates from 16 to 50% have been reported.[3]

In the present studies sera from 100 healthy South Florida donors (52 female and 48 male) were evaluated in the *Is*-Toxoplasma IgG Test Kit. Of the 100 samples, 22 (22%) were found to be positive and 78 (78%) were negative. Age distribution, geographic location and prevalence is provided in Table 1. Histograms demonstrating the distribution of IU/ml values are shown in Figures 1 and 2.

Thirty-seven of the female donors were of child-bearing age (18-45 years). Of the sera from these donors, 2 (5%) were positive and 35 (95%) were negative. A total of 45 sera from pregnant females (15 from each trimester) were also tested in the *Is*-Toxoplasma IgG Test Kit. Nine (20%) were positive and 36 (80%) were negative for anti-toxoplasma IgG. In addition, a total of 216 samples from females of childbearing age were identified in the outside and in-house clinical studies (these included the 45 sera from pregnant females already referenced). Of these samples, 45 (21%) were positive and 171 (79%) were negative for anti-toxoplasma IgG when evaluated in the Toxoplasma IgG Test Kit.

TABLE 1

Total Number	Number of donors	Prevalence
	100	22%
Geographic Locations: SouthEastern US	100	22%
Age		
10 – 19	13	15.4%
20 – 29	23	17.4%
30 – 39	40	17.5%
40 – 49	13	15.4%
50 – 59	5	40.0%
60 - 69	6	83.0%

FIGURE 1
***Is*-Toxoplasma IgG Positive Population**

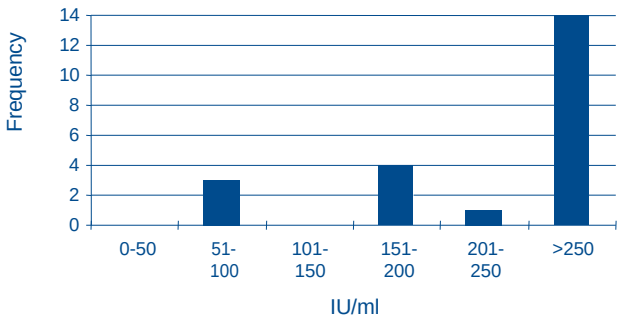
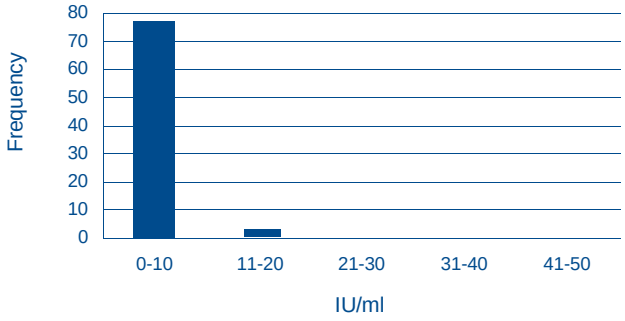


FIGURE 2
***Is*-Toxoplasma IgG Negative Population**



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of six hundred and twenty one sera were tested for the presence of Toxoplasma IgG antibodies using the Diamedix *Is*-Toxoplasma IgG Test Kit and three other marketed tests at two independent sites (site #1, Miami, FL and site #2, Salt Lake City, Utah) as well as at Diamedix Corp., Miami, FL (site #3). At site #3 testing was performed both manually and using the MAGO® Plus Automated EIA Processor.

Site #1 tested 200 samples (37% fresh and 73% frozen). Samples were obtained from the S. Florida area. Site #1 purposefully selected their sample population in order to provide an equal number of positive and negative results. Table 2 compares the results obtained for the *Is*-Toxoplasma IgG Test Kit and their currently used testing method and presents the overall agreement.

Site #2 tested 179 samples (all fresh) submitted for ToRCH screening. Samples were obtained from the West region. Table 3 compares the results obtained for the *Is*-Toxoplasma IgG Test Kit and their currently used testing method:

TABLE 2
***Is*-Toxoplasma IgG - Site #1**

		Positive	*Negative*	*Equivocal*
	Positive	98 [23]	14 [4]	3
Other	Negative	1	77 [29]	0
EIAs	Equivocal	0	6	1

		*95% CI**
Overall Agreement**	175/190 = 92.1 %	87.3 – 95.5

TABLE 3
Is-Toxoplasma IgG - Site #2

		Positive	*Negative*	*Equivocal*
	Positive	14 [6]	1	0
Other	Negative	0	164 [65]	0
EIAs	Equivocal	0	6	0

		*95% CI**
Relative Sensitivity	14/15 = 93.3%	68.0-99.8
Relative Specificity	164/164 = 100.0%	97.8-100.0
Overall Agreement**	178/179 = 99.4%	96.9 – 100.0

[ ] denotes samples from females of childbearing age

* 95% Confidence Intervals (CI) calculated by the Exact Method.[10]

** Equivocal results were excluded from calculations

Site #1, further resolution of the discordant samples was performed by testing such samples in a referee EIA method. Twelve of the samples negative by the *Is*-Toxoplasma IgG Test Kit and positive by the other EIA were negative by the referee method; the remaining two sera were equivocal. The sample that was positive in the Toxoplasma IgG Test Kit and negative in the other EIA was negative when tested by the referee method.

For Site #2, further resolution of the discordant sample was performed in a similar manner. The sample that was negative in the *Is*-Toxoplasma IgG Test Kit and positive by the other EIA was negative in the referee EIA method.

Site #3 (Diamedix Corp.) tested 242 samples (all frozen) by the manual method and 241 of these samples (one being QNS) by the MAGO Plus method. Samples were obtained from S. Florida blood donors. Tables 4 and 5 compare the results obtained for the *Is*-Toxoplasma IgG Test Kit and another marketed EIA method.

TABLE 4
Is-Toxoplasma IgG
Site #3: Manual

		Positive	*Negative*	*Equivocal*
	Positive	47 [16]	5	2
Other	Negative	1	186 [73]	0
EIAs	Equivocal	0	1	0

		*95% CI**
Relative Sensitivity	47/52 = 90.4%	79.0 – 96.8
Relative Specificity	186/187 = 99.5%	97.1 – 100.0
Overall Agreement**	233/239 = 97.5%	94.6 – 99.1

TABLE 5
Is-Toxoplasma IgG
Site #3: MAGO Plus

		Positive	*Negative*	*Equivocal*
	Positive	49	4	1
Other	Negative	1	185	0
EIAs	Equivocal	0	1	0

		*95% CI**
Relative Sensitivity	49/53 = 92.5%	81.8 – 97.9
Relative Specificity	185/186 = 99.5%	97.0 – 100.0
Overall Agreement**	234/239 = 97.9%	95.2 – 99.3

[ ] denotes samples from females of childbearing age (18-45 years)

* 95% Confidence Intervals (CI) calculated by the Exact Method.[10]

** Equivocal results were excluded from calculations

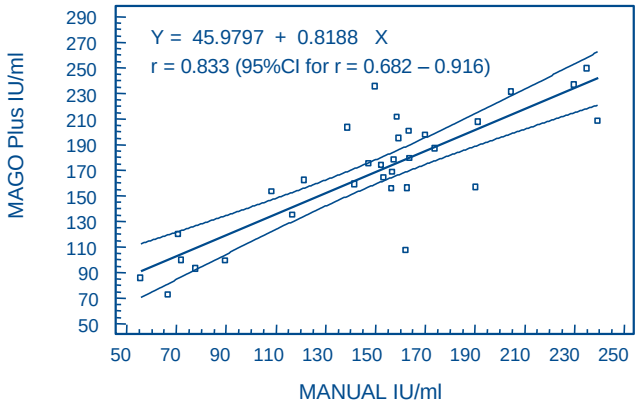
For Site #3 (manual testing), further resolution of the discordant sera revealed that the 5 sera negative in the *Is*-Toxoplasma IgG Test Kit but positive in the other EIA were also negative by a referee EIA method. The serum that was positive in the *Is*-Toxoplasma IgG Test Kit and negative in the other EIA was positive by the referee method. For MAGO Plus testing, the 4 sera that were negative in the *Is*-Toxoplasma IgG Test Kit but positive in the other EIA were also negative by a referee EIA method. The serum that was positive in the *Is*-Toxoplasma IgG Test Kit and negative in the other EIA was positive by the referee method.

NOTE : Please be advised that 'relative' refers to the comparison of the assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

B. Correlation of Manual and MAGO Plus Results

The *Is*-Toxoplasma IgG Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus Procedures, the results of 32 serum samples whose IU/ml results were within the assay's critical range (50 to 250 IU/ml) were compared. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in Figure 3.

FIGURE 3
Manual vs. MAGO Plus Correlation



C. Linearity

Several strongly positive serum samples were serially diluted and separate dilutions were assayed, in duplicate, in the *Is*-Toxoplasma IgG Test Kit both manually and using the MAGO® Plus Automated EIA Processor. Representative linear regression graphs and scattergrams of the mean results with 95% confidence intervals are presented in Figures 4 and 5 for one patient sample. The results demonstrate a high degree of linearity throughout the reportable range of the assay when samples are tested either manually or by MAGO Plus.

FIGURE 4
Manual Linearity

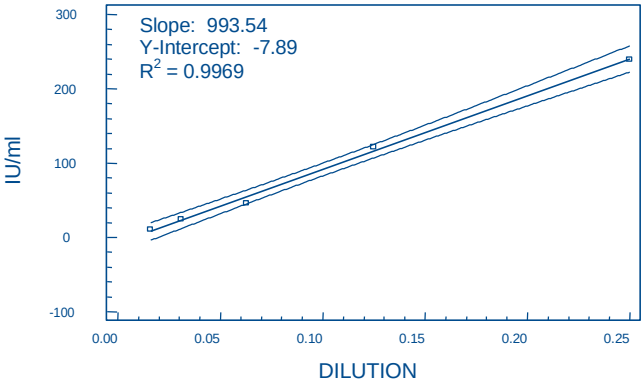
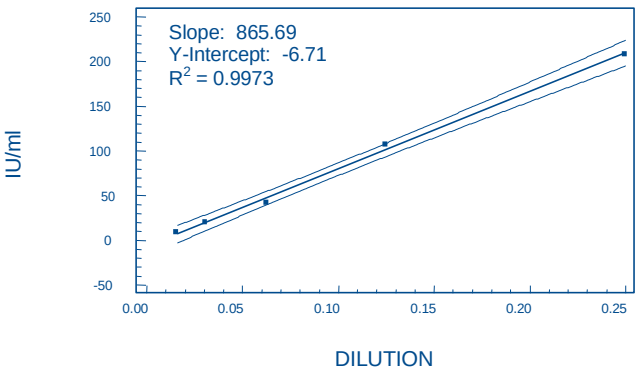


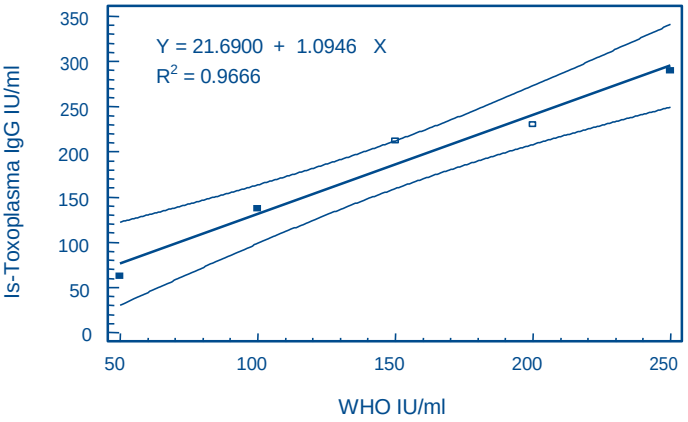
FIGURE 5
MAGO Plus Linearity



D. Correlation to WHO Standard

The *Is*-Toxoplasma IgG Test Kit has been calibrated against the WHO 3rd International Standard for Anti-Toxoplasma Serum (code TOXM). To demonstrate the accuracy of the quantitative procedure, several dilutions of the WHO Standard were prepared and assayed manually in triplicate in two different runs on two different days versus the *Is*-Toxoplasma IgG Test Kit standard curve. The linear regression graph and scattergram of the mean results with 95% Confidence Intervals is shown in Figure 6.

FIGURE 6
Dilutions of WHO Standard
assayed against *Is*-Toxoplasma IgG Standards



E. Semi-Quantitative Data

Serum pairs were obtained by preparing multiple two-fold dilutions of several strongly positive sera. Ratios for dilutions representing a four-fold and two-fold difference in antibody level were evaluated as a serum pair both manually and using the MAGO Plus. The overall mean ratio obtained for four-fold dilutions was 4.40 (SD 0.57) and the overall mean ratio obtained for two-fold dilutions was 2.19 (SD 0.27). Overall, it was estimated that a 3.8-fold or greater (mean ratio minus 1 SD) increase in *Is*-Toxoplasma IgG IU/ml ratio corresponded to a four-fold titer increase in Toxoplasma IgG antibody level. A ratio in the range of 2.2 to 3.8 was considered equivocal for significant increase determination.

F. Cross Reactivity

Sera containing IgG antibodies to viruses potentially cross-reactive to *T. gondii* have been tested in the *Is*-Toxoplasma IgG Test Kit. Fifty sera positive for one or more viruses and negative for IgG antibodies to *T. gondii* in the *Is*-Toxoplasma IgG Test Kit as well as in another marketed test were evaluated. In addition, nine of these sera were positive for anti-nuclear antibodies (ANA) and two were positive for anti-DNA. The data in the following table suggest that no cross-reactivity should be expected with the *Is*-Toxoplasma IgG Test Kit from these analytes.

Analyte	Toxoplasma IgG	VZV IgG	HSV IgG	CMV IgG	Rubella IgG	EBV IgG	anti-DNA	ANA
No. of Pos. Samples	0	48	47	37	49	48	2	9

G. Precision

Six serum samples, spanning the reportable range, as well as the 50 IU/ml kit Standard and kit Low Positive and Negative Controls were tested quantitatively and values calculated from IU/ml results. Sites #1 and #2 tested samples in triplicate in three separate runs on three different days. Site #3 (Diamedix Corp.) tested samples in triplicate in two separate runs on three different days both manually and using the MAGO® Plus Automated EIA Processor. The results obtained are shown in Tables 7-10.

TABLE 7 - Site #1
Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%
A	7.9	1.35	17.09	8.2	2.05	25.00	7.9	2.36	29.87	8.0	1.71	21.38
B	31.3	1.40	4.47	30.7	1.46	4.76	26.2	2.90	11.07	29.4	3.00	10.20
C	159.7	4.51	2.82	173.6	5.21	3.00	147.2	7.01	4.76	160.2	12.45	7.77
D	170.8	13.32	7.80	177.4	14.38	8.11	165.4	29.49	17.83	171.2	18.45	10.78
E	274.7	28.55	10.39	259.1	23.31	9.00	257.1	14.66	5.70	263.6	21.52	8.16
F	83.1	10.28	12.37	89.6	12.94	14.44	81.1	19.90	24.54	84.6	13.50	15.96
50 STD	54.6	4.46	8.17	54.9	5.60	10.20	53.5	8.35	15.61	54.3	5.53	10.18
LPC	128.1	1.56	1.22	138.1	3.15	2.28	146.4	18.42	12.58	137.7	12.16	8.83
NC	13.2	0.40	3.03	12.9	0.51	3.95	15.7	1.65	10.51	13.9	1.58	11.37

TABLE 8 - Site #2
Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%
A	10.1	1.46	14.46	5.4	1.03	19.07	1.1	1.47	133.60	5.5	4.07	74.00
B	25.9	3.56	13.75	25.0	2.79	11.16	21.0	1.15	5.48	24.0	3.25	13.54
C	145.1	8.32	5.73	136.3	5.15	3.78	70.3	4.79	6.81	117.2	35.83	30.57
D	162.8	8.92	5.48	132.9	3.70	2.78	95.1	2.96	3.11	130.3	29.81	22.88
E	250.0	0.00	0.00	237.9	9.96	4.19	189.2	8.92	4.71	225.7	28.68	12.71
F	56.8	6.61	11.64	75.5	2.76	3.66	31.0	3.80	12.26	54.5	19.77	36.28
50 STD	48.7	6.57	13.49	51.7	3.79	7.33	41.8	0.76	1.82	47.5	5.82	12.28
LPC	134.9	19.60	14.53	123.1	11.22	9.11	123.1	11.22	9.11	127.0	13.92	10.96
NC	11.8	1.68	14.24	11.5	1.93	16.78	11.5	1.93	16.78	11.6	1.61	13.88

TABLE 9 - Site #3
Intra-Assay and Interassay Precision (Manual)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%
A	2.6	1.51	58.08	1.9	0.44	23.16	2.4	0.70	29.17	2.3	0.98	42.61
B	17.4	2.08	11.95	19.3	2.28	11.81	19.2	1.41	7.34	18.7	2.04	10.91
C	115.3	8.59	7.45	105.6	9.88	9.36	105.6	22.04	20.87	108.8	14.69	13.50
D	141.6	5.54	3.91	141.6	6.90	4.87	137.7	10.86	7.89	140.3	7.82	5.57
E	238.7	20.56	8.61	246.8	16.69	6.76	240.7	19.48	8.09	242.1	18.17	7.51
F	55.1	5.21	9.46	46.3	1.62	3.50	58.0	8.22	14.17	53.3	7.32	13.73
50 STD	56.7	8.12	14.32	55.4	8.99	16.23	57.3	3.53	6.16	56.5	6.89	12.19
LPC	138.8	10.99	7.92	139.0	8.07	5.81	148.6	8.29	5.58	142.1	9.87	6.95
NC	14.1	0.96	6.81	12.9	1.21	9.38	15.4	2.09	13.57	14.1	1.76	12.48

TABLE 10 - Site #3
Intra-Assay and Interassay Precision (MAGO Plus)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%	MEAN IU/ml	SD	CV%
A	2.0	1.54	77.00	2.0	2.60	130.00	3.9	1.89	48.46	2.6	2.15	82.69
B	17.9	2.34	13.07	12.3	8.70	70.73	20.3	6.22	30.64	16.8	6.87	40.89
C	150.0	5.99	3.99	154.3	5.07	3.29	156.6	20.31	12.97	153.6	12.14	7.90
D	149.5	20.43	13.67	163.8	19.35	11.81	181.6	16.15	8.89	165.0	22.19	13.45
E	>250	N/A	N/A	>250	N/A	N/A	>250	N/A	N/A	>250	N/A	N/A
F	40.9	1.95	4.77	40.4	2.42	5.99	41.8	2.65	6.34	41.0	2.30	5.61
50 STD	65.7	4.24	6.45	79.2	10.45	13.19	78.4	9.35	11.93	74.4	10.19	13.70
LPC	170.9	11.34	6.64	195.8	8.83	4.51	208.8	15.98	7.65	191.8	19.94	10.40
NC	15.8	2.18	13.80	15.1	4.82	31.92	24.8	3.52	14.19	18.5	5.70	30.81

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