Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

Toxoplasma IgM Capture
Enzyma Immunoassay Tast Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-310- 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of Cut-Off Calibrator, controls and patient samples in Sample Diluent. Mix Well.
2. Add 100 µl of diluted Cut-Off Calibrator, controls and patient samples into the wells.

   Reserve the first well for reagent blank (100 µl of Sample Diluent).
3. Incubate at 37 ± 3°C for 60 ± 5 min.
4. Prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent and 0.1 ml (100 µl) 30X Tracer to each vial of lyophilized antigen. Note that 1 vial of lyophilized antigen is sufficient for at least 2 strips.
5. After incubation, discard the contents of the wells. Wash the wells 3 times with Wash Solution.
6. Add 100 µl of prepared Enzyme Tracer to each well.
7. Incubate at 37 ± 3°C for 60 ± 5 min.
8. Wash the wells as in #5 above.
9. Add 100 µl Substrate Solution to each well.
10. Incubate at 37 ±3°C for 20 ± 2 min.
11. Add 100 µl Stop Solution to each well.
12. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the presumptive qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum by capture enzyme immunoassay. When performed in conjunction with an anti-*Toxoplasma gondii* IgG assay, the *Is*-Toxoplasma IgM Capture assay can be used as an aid in the presumptive diagnosis of acute, recent or reactivated *Toxoplasma gondii* infection. Performance has not been established in newborns. This assay has not been cleared/approved by the FDA for blood/plasma donor screening.

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 utaro*, 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 without the need for specially formulated diluents.

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.

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. The presence of 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. Enzyme 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.

REAGENTS

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

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

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Lyophilized Antigen	Six vials of lyophilized *Toxoplasma gondii* antigen, whole cell sonicate, strain RH.
30X Tracer	One vial with red cap containing 1.0 ml mouse monoclonal anti-*Toxoplasma gondii* conjugated to horseradish peroxidase (30X concentrate) in stabilizer.
Tracer Diluent	One bottle with red cap containing 30 ml borate buffer. Also includes protein stabilizers, gentamycin and Proclin® 300 as preservatives. Color-coded pink.
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.
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.

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.

WARNINGS & PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

1. Handle samples, calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the calibrator 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 in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. 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.
3. Never pipette by mouth.
4. Avoid contact with open skin and mucous membranes.
5. 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.
6. 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.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
9. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
10. Store unused reagents at 2 to 8°C.
11. Incubations above or below the recommended temperatures or times may give erroneous results.
12. 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.
13. Coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
14. *(Manual Procadura Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section.)

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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 (2-8°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. The CLSI, formerly NCCLS, provides recommendations for collecting and storing blood specimens.[12]

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.

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 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 diluted Calibrator, controls and patient samples, to the wells. Avoid formation of bubbles when transferring diluted samples.

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3. Allow the wells to incubate uncovered at 37 ± 3°C for 60 ± 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. Mix until all the lyophilized material is reconstituted. Then add 100 µl of 30 X Tracer to each antigen vial and mix well. Allow the prepared Enzyme Tracer to sit at room temperature (18-30°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 µl each of Wash Solution. After adding the 3rd 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 µl of Enzyme Tracer into each well (*axcapt tha 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 each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 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.

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QUALITY CONTROL

1. The Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.100.
3. The absorbance of the Cut-Off Calibrator must be ≥ 0.150 when read against the blank.
4. The Low Positive and Negative Control must be within their assigned ranges.

If any one of these criteria is not met, the results are invalid and the test should be repeated.

NOTES: Additional controls may be tested according to guidelines or requirements of local, state 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

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value for the Cut-off Calibrator, exclude any absorbance value that deviates by more than 20% from the mean of the three absorbance values. 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:

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

Exampla: Absorbanca valuas obtainad for tha Cut-Off Calibrator: O.356, O.345, O.368 (aftar subtraction of tha Blank)

Maan Absorbanca of tha Cut-Off Calibrator = O.356

Sampla Absorbanca = O.959

Indax Valua = 2.69

When using an Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor), results are calculated and expressed as Positive, Equivocal or Negative.

2. Interpretation of Results

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 Results.

Index Value	Interpretation
< 0.90	Negative for anti-*Toxoplasma gondii* IgM
0.90 – 1.09	Equivocal for anti-*Toxoplasma gondii* IgM*
≥ 1.10	Presumptive positive for anti-*Toxoplasma gondii* IgM

Note that 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 at least fourteen days later and tested in parallel with the initial specimen. If the second sample remains 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.

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

3. Reporting Results

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

4. Predictive Values

The predictive value of a positive result decreases as the prevalence decreases. Interpretation of positive results in a low risk population should thus be made with caution. For example, the positive predictive value for a test which claims sensitivity and specificity of 95% would be 67.9% if the disease prevalence was 10% but would be only 16% if the disease prevalence was 1%.

5. General Guidelines for Interpretation of anti-*Toxoplasma gondii* results

Anti-T. gondii IgM result	*Anti-T. gondii IgG result*	*Report/Interpretation (excluding infants)*
Negative	**Negative**	It is presumed the patient has not been infected with and is not undergoing an acute infection with *Toxo-plasma gondii*. If symptoms persist submit a new specimen within three weeks.
Negative	**Positive**	From this testing it cannot be determined whether the patient is or is not undergoing a reactivated *Toxo-plasma gondii* infection. It appears the patient has been previously infected with *Toxoplasma gondii.* Infection occurred more than one year ago.
Negative	**Equivocal**	Obtain a new specimen for further testing. Patient may not be undergoing an acute infection with *Toxoplasma gondii.* Determining whether the patient has been previously infected with *Toxoplasma gondii* is not possible.
Equivocal	**Negative**	Obtain a new specimen for determination of IgM antibodies to *Toxoplasma gondii* . It cannot be deter-mined if the patient is undergoing an acute *Toxo-plasma gondii* infection. It appears the patient has not been previously infected with *Toxoplasma gondii*. If the new specimen result is positive or equivocal for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal	**Positive**	Obtain a new specimen for determination of IgM ant-ibodies to *Toxoplasma gondii.* It cannot be determined if the patient is undergoing or has undergone an acute *Toxoplasma gondii* infection. It appears the patient has been previously infected with *Toxoplasma gondii.* If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal	**Equivocal**	Obtain a new specimen for further testing. It cannot be determined if the patient is undergoing an acute infection or has been previously infected with *Toxo-plasma gondii.* If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	**Negative**	Obtain a new specimen for further testing. The patient may or may not be acutely infected with *Toxoplasma gondii* . Since the IgG antibodies to Tox*oplasma gondii* are negative, the specimen may have been obtained too early in the disease process for an accurate deter-mination. Retest the new specimen with a different anti-*Toxoplasma gondii* IgM assay. If the new speci-men result is still positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	**Positive**	The patient may or may not be acutely infected with *Toxoplasma gondii* . Obtain a new specimen for further testing. Since the IgG antibodies to *Toxoplasma gondii* are positive, it appears the patient may be acutely infected with *Toxoplasma gondii.* The new specimen should be repeated with a different anti-toxoplasma IgM assay. If the new specimen result is still positive for IgM and IgG antibodies to *Toxoplasma gondii* , the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	**Equivocal**	It cannot be determined if the patient is acutely infected with *Toxoplasma gondii.* Obtain a new specimen for further testing. Determining whether the patient has been previously infected with *Toxoplasma gondii* is not possible. The specimen may have been collected too early during the disease process for an accurate determination. Retest the new specimen with a dif-ferent anti-*Toxoplasma gondii* IgM assay. If the new specimen result is still positive for IgM and the IgG is positive/negative/equivocal for antibodies to *Toxo-plasma gondii* the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-Toxoplasma IgM Capture Test Kit cut-off value has been established to optimally discriminate those individuals with, from those without, IgM antibodies to *Toxoplasma gondii*. The optimal cut-off value was determined by statistical analysis of 200 normal sera shown to be negative for Toxoplasma IgM antibodies in other test methods. The mean and standard deviation of the absorbance values for these sera were 0.0918 and 0.0483 respectively. The cut-off was determined as being equal to the mean plus 3 standard deviations, 0.0918 + (3 X 0.0483) = 0.237. The Cut-Off Calibrator has been titrated to equal this result. Therefore, the mean value of the Cut-off Calibrator will be equal to the cut-off for the assay. To account for the inherent variation in EIA methods, an equivocal range of +/-10% has been included.

The cut-off value was further verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to two hundred and twenty sera assayed manually by Diamedix using the *Is*-Toxoplasma IgM Capture Test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-Toxoplasma IgM Capture Test Kit when performed manually has a relative sensitivity of 93% and a relative specificity of 98% based on comparison to the marketed test. Comparable values were obtained for MAGO Plus results.

The appropriateness of the cut-off was further confirmed using the CDC Toxoplasma 1998 Human Serum Panel (see under Performance Characteristics).

LIMITATIONS

1. The results obtained with the *Is*-Toxoplasma IgM Capture Test Kit serve only as an aid to diagnosis and should be used in conjunction with information available from the patient clinical evaluation and other available diagnostic procedures.
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 pretest 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 the MAGO Plus Automated EIA Processor have not been established.

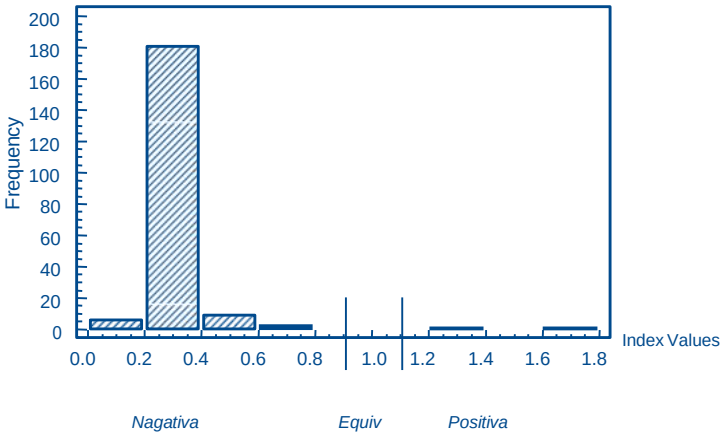
EXPECTED VALUES

The prevalence of Toxoplasma infection can vary depending on a number of factors such as age, gender, geographical location, socioeconomic status, race, type of test used, specimen collection and handling procedures, and clinical and epidemiological history of individual patients. The prevalence of Toxoplasma infection in the USA is in the range of less than 1 to 3%.[13, 14,15] In the present study two hundred sera from S. Florida blood donors were evaluated in the *Is*-Toxoplasma IgM Capture Test Kit. Of these samples, one hundred and ninety-eight (99%) were negative and two (1%) were positive. TABLE 1 shows the age and prevalence profile of this population. FIGURE 1 shows a distribution of Index values obtained for this population.

TABLE 1

	Number of Donors	% Seronegative	% Seropositive	% Equivocal
Total Number	200	99.0% (198)	1.0% (2)	0.0% (0)
Geographic Location:				
S. Florida	200			
Age				
10 – 19	18	100% (18)	0.0% (0)	0.0% (0)
20 – 29	47	97.9% (46)	2.1% (1)	0.0% (0)
30 – 39	74	100.0% (74)	0.0% (0)	0.0% (0)
40 – 49	40	97.5% (39)	2.5% (1)	0.0% (0)
50 – 59	11	100.0% (11)	0.0% (0)	0.0% (0)
60 – 69	9	100% (9)	0.0% (0)	0.0% (0)
>70	1	100.0% (1)	0.0% (0)	0.0% (0)
Gender				
Male	98	99.0% (99)	1.0% (1)	0.0% (0)
Female	102	99.0% (99)	1.0% (1)	0.0% (0)

FIGURE 1
***Is*-Toxoplasma IgM Results in a Normal Population**



(Note that the magnitude of the Index Value has no significance)

PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of four hundred and sixty-two sera were tested for the presumptive presence of Toxoplasma IgM antibodies using the Diamedix *Is*-Toxoplasma IgM Capture Test Kit and other legally marketed tests at two independent sites (site #1, California and site #2, New York) 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. Toxoplasma IgG antibody data was also available for a number of the samples tested.

Site #1, a large commercial clinical laboratory in California, not affiliated with the manufacturer, tested 121 samples. These samples consisted of 101 fresh samples submitted to the laboratory for Toxoplasma IgM antibody testing as well as 20 frozen samples with positive IgM antibody status. Samples were obtained nationwide. For the fresh samples, 32 were from males and 69 from females with ages ranging from 3 days to 66 years old. Of the samples tested 19 of the 20 frozen positive samples were also positive for Toxoplasma IgG antibodies. Of the 101 fresh samples 71 had also been tested for Toxoplasma IgG. Of these, 10 were positive and 61 were negative. TABLE 2 shows the results obtained for the *Is*-Toxoplasma IgM Capture Test Kit and their currently used IFA testing method. This table also denotes the Toxoplasma IgG results for the samples.

Site #2, a commercial reference laboratory in New York, not affiliated with the manufacturer, tested 121 samples. These samples consisted of 50 fresh samples and 50 frozen samples submitted to the laboratory for Toxoplasma IgM screening. Samples were obtained from various regions. This sample population was supplemented with 21 frozen samples procured from a vendor based on their positive serostatus. This positive serostatus was based on the results of another IgM test and not on documented clinical disease. Twenty-eight of the samples were from males and 79 from females with the remainder unidentified as regards gender. Of the female population (non-vendor samples) 52 were identified as prenatal samples. Patient ages ranged from 3 days to 80 years old. Toxoplasma IgG data was available for vendor samples. TABLE 3 shows the results obtained for the *Is*-Toxoplasma IgM Capture Test Kit and their currently used EIA testing method. TABLE 3a shows the comparative results for the prenatal samples only.

TABLE 2
Is-Toxoplasma IgM Capture - Site #1

		Positiva	*Nagativa*	*Equivocal*
	Positive	21 [20/20]	5 [2/5]	0
IFA	Negative	0	94 [10/71]	1
	*Equivocal	0	0	0

		´´95% CI
Overall Agreement	115/120 = 95.8%	90.5 – 98.6

TABLE 3
Is-Toxoplasma IgM Capture - Site #2

		Positiva	*Nagativa*	*Equivocal*
	Positive	7 [6/21]	5 [5/21]	5 [5/21]
Other	Negative	1	102	0
EIA	*Equivocal	1	0	0

		´´95% CI
Overall Agreement	109/115 = 94.8%	89.0 – 98.1

TABLE 3a – Prenatal Samples
Is-Toxoplasma IgM Capture - Site #2

		Positiva	*Nagativa*	*Equivocal*
	Positive	0	0	0
Other	Negative	0	52	0
EIA	*Equivocal	0	0	0

		´´95% CI
Overall Agreement	52/52 = 100%	93.2 – 100.0

*Equivocal results were excluded from calculations

** 95% Confidence Intervals (CI) calculated by the Exact Method.[11]

[ ] denotes number of samples positive for IgG / number tested for IgG

For Site #1, the 5 samples that were negative in the *Is*-Toxoplasma IgM Capture Test Kit and positive by IFA all had titers of 1:20, the minimum positive titer. Further testing of these discordant samples was performed by assaying them using a referee capture EIA method. Two of the samples were negative, two were equivocal and one weakly positive when tested with a referee capture EIA method.

For Site #2, further testing of the six discordant samples was performed in a similar manner. Of the 5 samples negative in the *Is*-Toxoplasma IgM Capture and positive by the other EIA, four were weakly positive and one was negative in a referee EIA capture method. The sample that was positive in the *Is*-Toxoplasma IgM Capture and negative by the other EIA was negative in the referee EIA capture method. All discrepancies occurred with the samples procured from a vendor.

Site #3 (Diamedix Corp.) tested 220 samples (all frozen) by the manual and MAGO Plus methods. Of these samples 111 were obtained from the normal S. Florida blood donor population. In addition, ninety-nine defined seropositive samples were obtained from a hospital located in Italy specializing in the prevention of congenital diseases. Ninety-seven of these samples were also positive for IgG antibodies. Of these samples, sixty-eight were from pregnant women (sixty-six of these samples were positive for IgG antibodies). The remaining ten samples were from a commercially obtained reference panel. All ten were positive for IgG antibodies. TABLE 4 show the results obtained for the normal population and TABLE 5 shows the results for the positive population using the *Is*-Toxoplasma IgM Capture Test Kit compared to another marketed capture EIA method. TABLE 5a shows the performance of the prenatal samples.

TABLE 4
Normal Population - Site #3: Manual
Is-Toxoplasma IgM Capture

		Positiva	*Nagativa*	*Equivocal*
Other	Positive	0	0	0
Capture	Negative	1	110	0
EIA	*Equivocal	0	0	0

		´´95% CI
Relative Specificity	110/111 = 99.1%	95.1 – 100.0
Overall Agreement	110/111 = 99.1%	95.1 – 100.0

TABLE 5
Positive Population - Site #3: Manual
Is-Toxoplasma IgM Capture

		Positiva	*Nagativa*	*Equivocal*
Other	Positive	93 [93]	3 [3]	6 [6]
Capture	Negative	1 [1]	6 [6]	0
EIA	*Equivocal	0	0	0

		´´95% CI
Overall Agreement	99/103 = 96.1%	90.4 – 98.9

TABLE 5a – Prenatal Samples
Is-Toxoplasma IgM Capture - Site #3

		Positiva	*Nagativa*	*Equivocal*
Other	Positive	60 [58]	2 [2]	3 [3]
Capture	Negative	1 [1]	2 [2]	0
EIA	*Equivocal	0	0	0

		´´95% CI
Overall Agreement	62/65 = 95.4%	87.1 – 99.0

*Equivocal results were excluded from calculations

** 95% Confidence Intervals (CI) calculated by the Exact Method.[11]

[ ] denotes number of samples positive for IgG

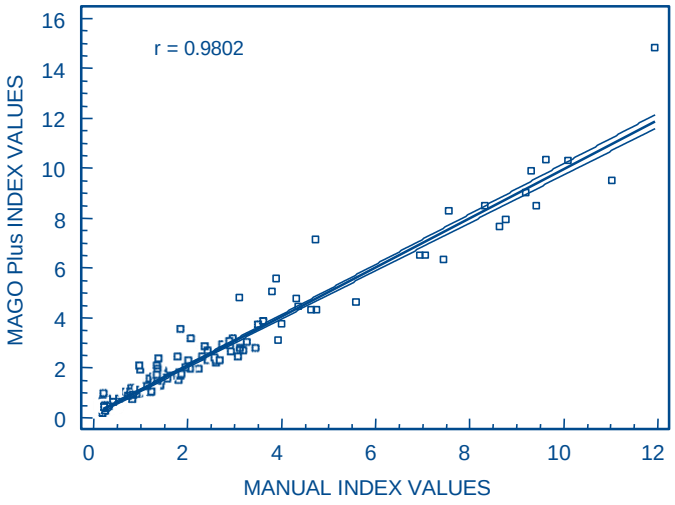
For Site #3, further testing of the 5 discordant sera revealed that the 3 sera negative in the *Is*-Toxoplasma IgM Capture Test Kit but positive in the other capture EIA were negative by a referee EIA method. The 2 sera that were positive in the *Is*-Toxoplasma IgM Capture Test Kit and negative in the other capture EIA were negative 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 IgM Capture Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the procedures, the results of the 220 samples tested manually and using the MAGO Plus were compared. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in FIGURE 2. The correlation coefficient (r) was 0.9802.

FIGURE 2
Manual vs. MAGO Plus Correlation



C. CDC Serum Panel Data

The following information is from a serum panel obtained from the CDC and tested by Diamedix. The results are presented as a means to convey further information on the performance of this assay with a masked characterized panel. This does not imply an endorsement of the assay by the CDC.

The panel consists of 32% true positive samples and 65% true negative samples. The Diamedix *Is*-Toxoplasma IgM Capture Test Kit demonstrated 99% total agreement with the CDC results. Of the results obtained by Diamedix there was 100% (32/32) agreement with the true positive specimens and 98.5% (64/65) agreement with the true negative specimens.

D. Cross-Reactivity / Interference Studies

The specificity of the *Is*-Toxoplasma IgM Capture Test Kit was validated by testing a number of sera containing relatively high levels of IgM antibody to other viruses as determined using commercially available test kits. A total of 26 known IgM positive sera were tested. In addition, the effect of potential interference from rheumatoid factor (RF), anti-nuclear antibody (ANA), viral-specific IgG and heterophile antibodies was assessed by testing an additional 23 sera. These data are shown in TABLE 6. TABLE 7 shows the lack of interference in samples containing high levels of IgG antibodies and low levels of IgM antibodies before and after the removal of the IgG.

TABLE 6

Specificity	# of Positive Samples	# Positive in the *Is*-Toxo IgM Capture
EBV IgM	8	0
Lyme IgM	3	0
CMV IgM	5	0
HSV IgM	5	0
Rubella IgM	5	0
Heterophile Antibody	4	0
RF	5	0
ANA	10	0
Toxoplasma IgG	4	0

TABLE 7

Sample #	Before IgG Removal		After IgG Removal	
	IgG Index	IgM Index	IgG Index	IgM Index
1	3.99	1.81	0.00	1.65
2	3.65	1.65	0.00	1.69
3	4.12	1.51	0.01	1.54
4	1.42	2.42	0.05	2.36
5	4.99	3.46	0.13	1.97
6	3.82	1.63	0.00	1.78
7	3.90	1.41	0.08	1.70
8	4.48	2.18	0.07	2.48
9	2.16	2.50	0.00	2.67
10	3.04	1.25	0.00	1.46

IgG Pos $\geq$ 1.00 IgM Pos $\geq$ 1.10

E. Verification of IgM Specificity

To confirm that the *Is*-Toxoplasma IgM Capture Test Kit specifically detects IgM-class antibodies, 13 samples with high levels of Toxoplasma IgM antibodies were selected for testing. These samples were treated with 20 mM dithiothreitol (DTT) to destroy the IgM and were then retested in the *Is*-Toxoplasma IgM Capture Test Kit. The results in TABLE 8 show that these samples were rendered negative when treated with DTT confirming the specificity of the *Is*-Toxoplasma IgM Capture test kit for detecting IgM-class antibodies.

TABLE 8

Sample #	Untreated		Treated with 20 mM DTT	
	Is-Toxo IgM Capture		Is-Toxo IgM Capture	
	Index	Interp	Index	Interp
1	6.53	POS	0.37	NEG
2	3.43	POS	0.27	NEG
3	9.60	POS	0.44	NEG
4	2.71	POS	0.26	NEG
5	5.47	POS	0.24	NEG
6	5.92	POS	0.24	NEG
7	7.51	POS	0.33	NEG
8	2.75	POS	0.20	NEG
9	1.73	POS	0.23	NEG
10	6.57	POS	0.29	NEG
11	2.84	POS	0.62	NEG
12	7.31	POS	0.29	NEG
13	4.43	POS	0.24	NEG

F. Precision

Six serum samples, as well as the kit Controls, were tested to assess the precision of the *Is*-Toxoplasma IgM Capture Test Kit. 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 9-12.

TABLE 9
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
T1	0.211	0.018	8.53	0.187	0.018	9.63	0.191	0.004	2.09	0.196	0.017	8.67
T2	0.241	0.006	2.49	0.229	0.013	5.68	0.261	0.019	7.28	0.244	0.018	7.38
T3	1.761	0.087	4.94	1.826	0.027	1.48	1.714	0.092	5.37	1.767	0.081	4.58
T4	1.668	0.049	2.94	1.942	0.069	3.55	1.815	0.042	2.31	1.808	0.128	7.08
T5	2.838	0.157	5.53	3.296	0.114	3.46	3.014	0.099	3.28	3.049	0.228	7.48
T6	3.496	0.062	1.77	3.866	0.066	1.71	3.498	0.081	2.32	3.620	0.194	5.36
LPC	1.390	0.035	2.52	1.650	0.042	2.55	1.450	0.015	1.03	1.497	0.121	8.08
NC	0.279	0.031	11.11	0.230	0.013	5.65	0.262	0.011	4.20	0.257	0.028	10.89

TABLE 10
Site #2- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
T1	0.296	0.053	17.91	0.416	0.072	17.31	0.181	0.029	16.02	0.297	0.112	37.71
T2	0.298	0.062	20.81	0.465	0.044	9.46	0.290	0.040	13.79	0.351	0.096	27.35
T3	1.796	0.054	3.01	1.449	0.024	1.66	1.896	0.047	2.48	1.714	0.207	12.08
T4	1.991	0.116	5.83	1.735	0.092	5.30	1.963	0.038	1.94	1.896	0.144	7.59
T5	3.312	0.134	4.05	2.528	0.224	8.86	3.388	0.086	2.54	3.076	0.435	14.14
T6	4.128	0.224	5.43	3.604	0.085	2.36	3.797	0.176	4.64	3.843	0.273	7.10
CAL	0.998	0.122	12.22	1.143	0.141	12.34	1.142	0.030	2.63	1.094	0.119	10.88
LPC	1.554	0.128	8.24	1.549	0.108	6.97	1.516	0.075	4.95	1.540	0.093	6.04
NC	0.260	0.044	16.82	0.429	0.080	18.65	0.241	0.037	15.35	0.310	0.102	32.90

TABLE 11
Site #3-Intra-Assay and Interassay Precision (Manual)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=18)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A	0.226	0.016	7.08	0.248	0.019	7.66	0.213	0.026	12.21	0.229	0.025	10.92
B	0.312	0.046	14.74	0.283	0.033	11.66	0.268	0.025	9.33	0.287	0.039	13.59
C	1.874	0.021	1.12	1.861	0.077	4.14	1.912	0.089	4.65	1.882	0.069	3.67
D	1.983	0.068	3.43	1.993	0.110	5.52	2.025	0.089	4.40	2.000	0.087	4.35
E	3.331	0.089	2.67	3.260	0.100	3.07	3.423	0.142	4.15	3.338	0.126	3.77
F	3.895	0.308	7.91	4.286	0.420	9.80	4.284	0.175	4.08	4.274	0.258	6.04
c/o CAL	1.080	0.037	3.43	1.028	0.026	2.53	1.051	0.043	4.09	1.053	0.040	3.80
LPC	1.661	0.061	3.67	1.703	0.120	7.05	1.740	0.072	4.14	1.701	0.089	5.23
NC	0.290	0.036	12.41	0.314	0.035	11.15	0.271	0.013	4.80	0.291	0.033	11.34

TABLE 12
Site #3- Intra-assay and Interassay Precision (MAGO Plus)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=18)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A	0.34	0.027	7.94	0.35	0.050	14.29	0.31	0.129	41.61	0.33	0.079	23.65
B	0.41	0.073	17.80	0.46	0.054	11.74	0.35	0.044	12.57	0.40	0.069	17.08
C	1.68	0.065	3.87	1.77	0.081	4.58	1.88	0.196	10.43	1.77	0.147	8.30
D	2.07	0.448	21.64	2.05	0.108	5.27	2.16	0.152	7.04	2.09	0.268	12.80
E	3.40	0.430	12.65	3.41	0.261	7.65	3.84	0.303	7.89	3.55	0.382	10.77
F	3.90	0.308	7.90	4.22	0.197	4.67	4.94	0.379	7.67	4.35	0.533	12.25
c/o CAL	1.16	0.094	8.10	1.02	0.067	6.57	1.15	0.126	10.96	1.11	0.113	10.16
LPC	2.11	0.543	25.73	1.71	0.206	12.05	2.00	0.456	22.80	1.94	0.436	22.49
NC	0.44	0.117	26.59	0.43	0.052	12.09	0.43	0.168	39.07	0.44	0.107	24.67

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.
11. Gardner, M. J. and Altman, D. G. 1986. Confidence Intervals Rather than Hypothesis Testing. Brit. Med. J. 292 : 746-750.
12. Procedures for the Handling and Processing of Blood Specimens: Approved Guideline - Second Edition NCCLS Document H18-A2, Vol.19, No. 21.1999.
13. Hofgartner, W. T., Swanzy, S. R., Bacina, R. M., Condon, J., Gupta, M., Matlock, P. E. Bergeron, D. L., Plorde, J. J. and Fritsche, T. R. 1997. Detection of Immunoglobulin G (IgG) and IgM antibodies to *Toxoplasma gondii*: Evaluation of Four Commercial Immunoassay Systems. J. Clin. Microbiol. 35(12):3313-3315.
14. Guerina, N.G., Hsu, H.W., Meissner, H. C., Maguire, J. H., Lynfield, R., Stechenberg, B., Abroms, I., Pasternack, M. S., Hoff, R., and Eaton, R. B. 1994. Neonatal Serologic Screening and Early Treatment for Congenital *Toxoplasma gondii* Infection. N. Engl. J. Med. 330(26) 1858-1863.
15. Karim, K. A. 1977. Toxoplasmosis in Greater Victoria. Can. Med. Assoc. J. 117:895-896.
16. Manual Guide – Safety Management No. CDC – 22, "Decontamination of Laboratory Sink Drains to Remove Azide Salts", Centers for Disease Control and Prevention, Atlanta, GA, April 30, 1976.

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