Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16
Created by:	Approved By:	Date:

FOR IN VITRO DIAGNOSTIC USE

1.0 INTENDED USE: For the qualitative and quantitative detection of human IgM antibodies to Toxoplasma in human serum by enzyme immunoassay, to aid in the diagnosis of Toxoplasma infection. A positive result is presumptive for the detection of anti-*Toxoplasma gondii* antibodies and presumptive for the diagnosis of acute, recent, or reactivated Toxoplasma gondii infection. Patient testing with anti-*Toxoplasma gondii* IgM antibody assay, must be accompanied by an anti-Toxoplasma gondii IgG antibody assay. The assay's performance characteristics have not been established for neonatal toxoplasmosis diagnosis. This assay has not been cleared / approved by the FDA for blood / plasma donor screening.

2.0 SUMMARY AND EXPLANATION OF THE TEST:

Serologic studies indicate that infection with Toxoplasma gondii, an intracellular parasite and the causative agent of toxoplasmosis, is fairly widespread in the population worldwide. For example, it has been estimated that 30 % of the population in the United States exhibits serological evidence of exposure to Toxoplasma gondii[1]. The organism can be transmitted during organ transplantation[2], by blood or leukocyte transfusion[3], contact with contaminated cat feces[4], or by ingestion of raw or undercooked meat from infected animals[5].

In adults the infection is usually asymptomatic, although symptomatic as well as fatal cases do occur. Symptoms range from swollen lymph nodes to those resembling infectious mononucleosis[1]. In children, the disease may affect the central nervous system and the viscera. Congenital infection also occurs, and toxoplasmosis is a significant cause of mortality and congenital malformation[6-10].

The diagnosis of toxoplasmosis is frequently assisted by serological methods. The demonstration of toxoplasma IgM antibodies is presumptive evidence of recent, active or reactivated infection or, in the case of newborns congenital infection. Because less than one percent of newborns are born with maternally transferred IgM, the presence of Toxoplasma specific IgM antibodies is presumptive evidence of toxoplasmosis[11].

The Toxoplasma IgM EIA test is intended for the detection of IgM antibodies to toxoplasma. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, or as International Units (IU/ml), which are traceable to the WHO Anti-Toxoplasma Serum, 3rd International Standard Preparation, 1994.

3.0 PRINCIPLE OF THE PROCEDURE:

Diluted samples are incubated in antigen-coated wells. Absorbents have been included in the Diluent to neutralize the affects of rheumatoid factor and anti-toxoplasma IgG antibody. Toxoplasma antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme-labeled antibodies to human IgM) is added and incubated. If IgM antibodies to toxoplasma are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the enzyme-labeled substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

4.0 REAGENTS

Coated Wells	Coated with sonicated Toxoplasma gondii antigen, Strain: RH. 12 eight-well strips.
Well Support	One.
Diluent*	25 ml (pink color). Phosphate-buffered saline with a protein stabilizer, and absorbents for rheumatoid factor and human IgG.
Calibrator 1*	0.3 ml. Human serum. Strongly reactive for toxoplasma IgM antibodies. Index and IU/ml values shown on vial label.
Calibrator 2*	0.3 ml. Human serum. Moderately reactive for toxoplasma IgM antibodies. Index and IU/ml values shown on vial label.
Positive Control*	0.3 ml. Human serum. Reactive for toxoplasma IgM antibodies. Index and IU/ml values shown on vial label.
Negative Control*	0.3 ml. Human serum. Nonreactive for toxoplasma IgM antibodies.
Conjugate	12 ml (green color). Goat anti-human IgM labeled with alkaline phosphatase (calf).
Substrate	12 ml. p-nitrophenyl phosphate.

Note: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

Wash Concentrate*	30 ml. Tris-buffered saline with Tween 20, pH 8.0. Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to 1 liter of distilled or deionized water.
Stop Reagent	12 ml. Trisodium Phosphate 0.5 M.

* Contains sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

5.0 WARNINGS AND PRECAUTIONS

5.1 For *in vitro* diagnostic use.

5.2 Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The

Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by FDA licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV 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, or latest edition.

5.3 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.
5.4 Avoid contact with open skin.
5.5 Never pipet by mouth.
5.6 Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build- up of metal-azide compounds.
5.7 R 21/22: Harmful in contact with skin and if swallowed.
5.8 S24/25 - 36/37/39: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection. For further information, refer to product SDS.
5.9 Do not interchange reagents from different reagent lots, except for Wash Concentrate, Substrate and Stop Reagent.
5.10 Do not use reagents beyond their stated expiration date.
5.11 Incubation times recommended in the Test Procedure section should be adhered to.
5.12 Unused Coated Wells should be kept in their resealable bag with dessicant, and stored in the refrigerator.
5.13 Do not smoke, eat, drink, or apply cosmetics in areas where plasma/serum samples are handled.

6.0 HANDLING AND PROCEDURAL NOTES

6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.
6.2 Do not use past the expiration date indicated on the kit.
6.3 Do not interchange components of one kit with those of another kit.

7.0 STORAGE INSTRUCTIONS

Store all reagents at 2 to 8° C in an upright position when not in use. Do not freeze reagents.

8.0 INDICATIONS OF DETERIORATION

8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.
8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9.0 SPECIMEN COLLECTION AND STORAGE

9.1 Sera should be separated from clotted blood.
9.2 If specimens are not tested within 8 hours, they should be stored at 2 to 8° C. for up to 48 hours. Beyond 48 hours specimens should be stored at -20° C. or below.
9.3 Multiple freeze-thaw cycles should be avoided.
9.4 Samples containing visible particulate matter should be clarified by centrifugation; and grossly contaminated samples should not be used.
9.5 Samples should not be heat-inactivated before testing.

10.0 PERFORMANCE OF TEST

Materials Provided:

96 Tests

Coated Wells	12 eight-well strips	Positive Control	0.3 ml
Well Support	1	Negative Control	0.3 ml
Diluent	25 ml	Conjugate	12 ml
Calibrator 1	0.3 ml	Substrate	12 ml
Calibrator 2	0.3 ml	Stop Reagent	12 ml
		Wash Concentrate	30 ml

Additional Materials Required:

1. Microplate washer
2. Pipettors for dispensing 8, 100 and 200 µl
3. Timer

Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm. Dual or single wavelength readers may be used. Data on file.

11.0 TEST PROCEDURE

Preparation for the Assay

11.1 Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

11.2 Prepare 1:26 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the test set Diluent. For example: add 8 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.
Note: For qualitative assays, a single Calibrator may be used; for quantitative assays, use Calibrator 1 and Calibrator 2.

12.0 ASSAY PROTOCOL

12.1 Place an appropriate number of Coated Wells in the Well Support.
Note: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.

12.2 Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.
Note: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results.

12.3 Incubate the wells at room temperature (20 to 25° C.) for 30 ± 5 minutes.

12.4 Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.5 Place 2 drops (or 100 µl) of Conjugate into each well.

12.6 Incubate the wells at room temperature for 30 ± 5 minutes.

12.7 Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.8 Place 2 drops (or 100 µl) of Substrate into each well.

12.9 Incubate at room temperature for 30 ± 5 minutes.

12.10 Place 2 drops (or 100 µl) of Stop Reagent into each well.

12.11 Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.
Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

13.0 QUALITY CONTROL

Quality control requirements must be performed in accordance with applicable local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control Procedures. Controls with graded reactivity should be included. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the kit and contact ASI Technical Support at 800-654-0146.

14.0 INTERPRETATION OF RESULTS

Calculation of Results

Qualitative results may be calculated using a single calibrator. For quantitative results, use a calibration curve consisting of two or more calibrators.

Single Calibrator (Calibrator 2)

Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator Index or IU/ml}}{\text{Calibrator Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve

Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point) and the reagent blank (zero / origin), using a point-to-point curve fit. The upper range of the curve may be expanded by adding additional points. For example: the concentration of Calibrator 1 may be increased 1.5-fold, and 2-fold, by adding 12 µl and 16 µl of Calibrator 1 to 200 µl of the test set Diluent, and transferring 100 µl of each dilution to coated wells. The Index, or IU/ml values, assigned to these points, should be 1.5 and 2 times respectively, the value shown on the Calibrator 1 label. The extent to which the upper range of the standard curve may be expanded, will be limited by the calibrator(s) being used.

Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

Test Validation Criteria

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be at least 0.5 when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Negative Control must have an Index value less than 0.9, or an IU/ml value less than 370.
5. The Positive Control must have an Index value, or an IU/ml value, within the range printed on the label. When performing qualitative tests, users may supply an alternative Positive Control if they wish.
6. To validate the upper range of the assay when performing the quantitative procedure, the Positive Control should be run at higher concentrations. For example, the Positive Control should be assayed at 1.5-fold and 2-fold concentrations by adding 12 µl and 16 µl of the Positive Control, to 200 µl aliquots of the test set Diluent, and transferring 100 µl of each of these dilutions to coated wells. The expected value ranges for these concentrated controls would be 1.5 times and 2 times respectively, the expected value ranges printed on the Positive Control label. If the control values do not fall within the specified ranges, the assay is invalid and should be repeated. Optionally, users may supply alternative positive controls if they wish. If any of these criteria are not met, the test is invalid and should be repeated.
7. The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to NCCLS document C24-A, *Internal Quality Control Testing: Principles and Definitions.*

Interpretation of Results

Index Value	IU / ml	Interpretation
< 0.9	< 370	Negative
> 0.9 < 1.1	≥ 370 < 455	Equivocal
≥ 1.1	≥ 455	Presumptive Positive

The Toxoplasma IgM EIA cut-off values were based on statistical analyses, i.e. mean + 2 standard deviations, of 111 normal serum specimens, including 45 from women of childbearing age (18 to 45 years, mean age: 32). They were challenged in tests of known positive and negative specimens (see Performance Characteristics).

Specimens which yield absorbance values above the range of the test set calibrator(s), may be pre-diluted in the test set Diluent and reassayed. The resulting Index value must be multiplied by the dilution factor. Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.

Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgM level cannot be correlated to an endpoint titer. When the assay is used qualitatively, the magnitude of results above the cut-off is not an indicator of total antibody present.

Specimens collected too early during the course of the disease may not contain anti-toxoplasma IgM antibody. Furthermore, some individuals may not produce a detectable IgM response to toxoplasma infection.

Patient testing with an anti-Toxoplasma IgM antibody assay must be accompanied by an anti-Toxoplasma IgG antibody assay. The following table is consistent with the NCCLS' recommendation contained in: Specifications for Immunological Testing for Infectious Diseases; Approved Guideline (I/LA18- A).

Anti-T. gondii IgM Result	Anti-T. gondii IgG Result	Report / Interpretation
Negative	Negative	It is presumed the patient has not been infected with and is not undergoing an acute infection with Toxoplasma 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 Toxoplasma 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 determined if the patient is undergoing an acute Toxoplasma 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 antibodies 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 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.

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 Toxoplasma gondii are negative, the specimen may have been obtained too early in the disease process for an accurate determination. Retest the new specimen with a different anti-Toxoplasma gondii IgM assay. If the new specimen 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. 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 different 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 Toxoplasma gondii the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

15.0 LIMITATIONS OF THE PROCEDURE

The results obtained with the Toxoplasma IgM EIA test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Negative results do not rule out the diagnosis of toxoplasmosis. The specimen may be drawn before the appearance of detectable antibodies. Negative results in suspected early toxoplasmosis disease should be repeated in 4 to 6 weeks.

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.

The prevalence of anti-toxoplasma IgM antibodies will affect the assay's predictive value. Performance characteristics have not been determined with neo-natal or cord blood.

The performance characteristics of the Bio-Rad Toxoplasma IgM for any matrix other than serum have not been established.

The performance characteristics of this test with pre-transplant and transplant, or other immunosuppressed patients have not been established. The assay's performance characteristics have not been established for visual result interpretations.

Titration experiments have shown that the range of linearity for Toxoplasma IgM EIA index values is 1.1 to 5.5, or 455 to 2,500 for IU/ml values.

The absorbents in the Toxoplasma IgM EIA specimen diluent have been shown to block, or neutralize, up to 1,900 IU/ml of anti-toxoplasma IgG antibody, in combination with up to 448 IU/ml of IgM rheumatoid factor (RF).

Nevertheless, the possibility of positive reactions due to the presence of anti-toxoplasma IgG and IgM RF cannot be ruled out entirely.

16.0 EXPECTED VALUES

The prevalence of toxoplasma IgM antibodies is related to age and geographic location of the test population.

The incidence of anti-toxoplasma IgM antibody in normal, asymptomatic donors, has been reported to range from 0.9 % to 6 % [13, 14]. In a study[15] of clinically diagnosed toxoplasmosis, 100 % of the patients exhibited the presence of anti-toxoplasma IgM during the first month of illness. The seropositivity declined to 86 % during the second month, to 62 % during the third month, and to 54 % in the fourth month. Some individuals remained seropositive up to 2 years[15].

Studies performed with specimens obtained in the U.S., United Kingdom and South America, using the Toxoplasma IgM EIA test, revealed that the incidence of anti-toxoplasma IgM antibody among normal, asymptomatic donors in Miami, FL was four in one hundred and eleven, or 2.7 % (Table 1). In patients diagnosed with apparent toxoplasma infection, the incidence of anti-toxoplasma IgM was 42.2 % in Memphis, TN, and 77.8 % in the United Kingdom. The incidence of toxoplasma IgM in specimens obtained from HIV patients in the Memphis area was 5.6%.

Table 1. Results of Tests of 111 Archival Specimens (frozen), from Normal South Florida Donors, Including 55 from women of childbearing age. The Assays were Performed at Laboratory C (Miami, FL), Using the Toxoplasma IgM EIA Test.

IU/ml Value Ranges	Index Value Ranges	Specimens	
< 455	< 1.1	107 {52}	96.3 %
≥ 455 to < 1000	≥ 1.1 to < 2.4	3 {3}	2.7 %
≥ 1000 to < 2000	≥ 2.4 to < 4.8	1	0.9 %
≥ 2000	≥ 4.8	0	0 %

{ } Number of female donors of childbearing age.

Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

One hundred and three specimens, from women being tested prenatally for anti-toxoplasma IgM antibodies, were identified in the clinical studies. Of these, 28 (27.2 %) were positive, 10 (9.7 %) were equivocal, and 65 (63.1 %) were negative, when tested by the Toxoplasma IgM EIA test. The ranges of values obtained for these women are shown in Tables 2A and 2B.

Table 2A. Results of Toxoplasma IgM EIA tests of 75 Serum Specimens, Which Were Obtained from Women Being Tested Prenatally for Anti- toxoplasma IgM Antibody in Bogota, Colombia (Laboratory A).

IU/ml Value Ranges	Index Value Ranges	Specimens	
< 455	< 1.1	49	65.3 %
≥ 455 to < 1000	≥ 1.1 to < 2.4	23	30.7 %
≥ 1000 to < 2000	≥ 2.4 < 4.8	3	4.0 %
≥ 2000	≥ 4.8	0	0 %

Table 2B. Results of Toxoplasma IgM EIA tests of 28 Serum Specimens, Which Were Obtained from Women Being Tested Prenatally for Anti- toxoplasma IgM Antibody in Memphis, TN (Laboratory B).

IU/ml Value Ranges	Index Value Ranges	Specimens	
< 455	< 1.1	26	92.9 %
≥ 455 to < 1000	≥ 1.1 to < 2.4	2	7.1 %
≥ 1000 to < 2000	≥ 2.4 < 4.8	0	0 %
≥ 2000	≥ 4.8	0	0 %

15.0 PERFORMANCE CHARACTERISTICS

Comparative Testing

The results of Toxoplasma IgM EIA tests correlate well with other commercial serological tests. Serum specimens obtained from asymptomatic normal donors, acute toxoplasmosis patients, pregnant women, HIV patients, and patients with other conditions unrelated to toxoplasma infection, were assayed for the presence of anti-toxoplasma antibody using the Toxoplasma IgM EIA test and two other commercial serological assays. The assays were performed at two independent laboratories (Lab A, Bogota, Colombia and Lab B, Memphis, TN), and at Laboratory C (Miami, FL). The results obtained in these studies are shown below in Tables 3, 4 and 5, respectively.

Table 3. Results of Tests of 75 Archive Specimens Obtained from Pregnant Women in Bogota, Colombia, Performed at Laboratory A (Bogota, Colombia), Using the Toxoplasma IgM EIA Test and Another Commercial Test.

Comparative	Toxoplasma IgM EIA				
Test #1	Positive	Equivocal	Negative		95%CL**
Positive	24 {24}	1 {1}	6 {6}	Relative sensitivity*	65.7 to 94.3
Equivocal	1 {1}	6 {6}	3 {3}		
Negative	0	2 {2}	32 {32}	Relative specificity*	90.8 to 100
* Excluding equivocal results				Overall Agreement*	83.0 to 97.7

** Calculated by the Normal Method (16).

{ } Number of specimens obtained from pregnant women.

Table 4. Results of tests of 150 Specimens (73% frozen and 27% fresh),Obtained from Acute Toxoplasmosis Patients, Pregnant Women, Newborns, HIV Patients, and Other with Conditions Unrelated to Toxoplasma Infection, in Memphis, TN. The Tests were Performed at Laboratory B (Memphis, TN), Using the Toxoplasma IgM EIA Test and Another Commercial Test.

Comparative	Toxoplasma IgM EIA				95%CL**
Test #2	Positive	Equivocal	Negative		
Positive	10 {1}	2	9 {1}	Relative sensitivity*	30.2 to 75.1
Equivocal	0	0	0		
Negative	2 {1}	4 {1}	123 {24}	Relative specificity*	96.2 to 100
* Excluding equivocal results				Overall Agreement*	88.0 to 96.7

** Calculated by the Normal Method (16).

{ } Number of specimens obtained from pregnant women.

Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

Table 5. Results of tests of 128 Specimens (100% frozen) Which were Obtained in the U.S., United Kingdom and Colombia, The Specimens were Obtained from Normal Asymptomatic Donors, Acute Toxoplasmosis Patients, and Pregnant Women. The Tests were Performed at Laboratory C (Miami, FL), Using the Toxoplasma IgM EIA Test and Another Commercial Test.

Comparative	Toxoplasma IgM EIA				
Test #1	Positive	Equivocal	Negative		95%CL**
Positive	51 {26}	4	10	Relative sensitivity*	74.3 to 92.9
Equivocal	1 {1}	0	2		
Negative	4 {2}	1 {1}	55 {8}	Relative specificity*	86.8 to 99.6
* Excluding equivocal results				Overall Agreement*	82.6 to 94.1

** Calculated by the Normal Method (16).

{ } Number of specimens obtained from pregnant women.

The study specimens do not represent a normal U.S. population, inasmuch as they were selected to ensure that the reportable ranges of the devices were adequately covered, and that an adequate number of normal asymptomatic, acute toxoplasmosis and prenatal donors were present.

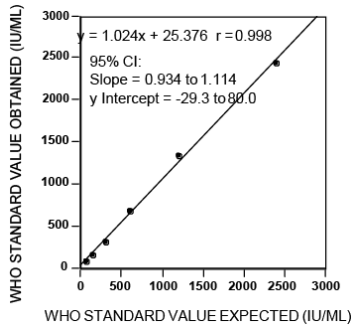
The acute toxoplasmosis patients in the study groups presented in Tables 4 and 5 (above), were tentatively diagnosed as having toxoplasmosis, and were confirmed by single-point serological testing at the study site.

Please be advised that "relative" sensitivity and specificity refers to the comparison of this assay's results to that of a similar assay. No judgement can be made on the comparison assay's accuracy to predict disease.

WHO Standard

The Toxoplasma IgM EIA test has been standardized against the WHO Anti-Toxoplasma Serum, 3rd International Standard Preparation, 1994. The recovery of the WHO Anti-Toxoplasma standard, using the Toxoplasma IgM EIA manual method, with the secondary standard, is plotted below in Figure 1.

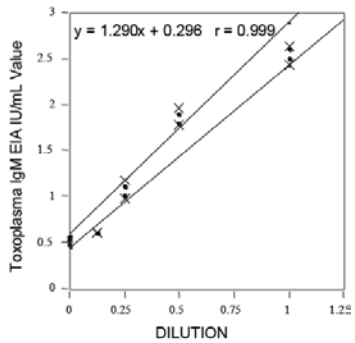
Figure 1. Recovery of the WHO Anti-Toxoplasma Serum, 3rd International Standard Preparation, Using the Secondary Standard and the Toxoplasma IgM EIA Test.



Titration curve

Several strongly positive serum specimens were serially diluted (two-fold) in triplicate, and assayed by the Toxoplasma IgM EIA test. Typical results are shown in Figure 2.

Figure 2. Titration Curve for a Strongly Positive Specimen.



Subject/Title: **ASI TOXOPLASMA IgM EIA TEST**		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

The triplicate data for each dilution are shown as points, the 95 % confidence limits for each set of triplicate data are indicated by (x's), and the 95 % confidence limits for the slopes and y-intercepts are represented by straight lines. The formula for the linear regression for the triplicate data is shown in Figure 2.

Specificity

Of fifty-eight specimens which were unreactive in the Toxoplasma IgM EIA test, 5 were shown to contain moderate to high levels of IgM antibody directed against cytomegalovirus, 6 against varicella zoster virus, 7 against Epstein-Barr virus (VCA), 5 against herpes simplex virus, 5 against rubella virus, 10 against type A influenza virus, 10 against measles, and 10 against parvovirus.

Precision

Eight serum specimens (2 negative and 6 positive) and the Toxoplasma IgM EIA positive and negative controls, were assayed in triplicate, on three separate occasions.

The precision experiments were performed manually at two independent laboratories (Lab A and Lab B) and at Laboratory C. These results are shown below in Tables 6, 7 and 8 respectively.

Table 6. Results Intra-assay and Interassay Precision Tests Performed at Lab A. Values were calculated from Toxoplasma IgM EIA Index and IU/ml Values.

	INTRA-ASSAY						INTERASSAY					
SAMPLE	MEAN INDEX	S.D.	C.V.%	MEAN IU/ml	S.D.	C.V.%	MEAN INDEX	S.D.	C.V.%	MEAN IU/ml	S.D.	C.V.%
Pos. Control	2.5	0.153	6.2	1246	122	9.8	2.4	0.156	6.6	1108	152	13.8
Neg. Control	0.2	0	NA	83	4	NA	0.2	0.053	NA	107	26	NA
1	0.4	0	NA	153	4	NA	0.4	0.053	NA	177	25	NA
2	0.3	0	NA	105	11	NA	0.3	0.033	NA	125	26	NA
3	1.4	0.153	10.7	498	47	9.5	1.5	0.132	9.0	527	52	9.8
4	1.7	0.208	12.5	610	86	14.1	1.8	0.183	10.1	663	92	14.0
5	1.7	0.115	6.9	586	27	4.7	1.7	0.112	6.6	613	33	5.3
6	1.5	0	0	536	15	2.8	1.6	0.192	11.8	599	91	15.1
7	1.8	0.153	8.3	687	117	17.0	1.9	0.172	9.1	703	91	13.0
8	2.4	0.115	4.7	1202	119	9.9	2.8	0.387	13.9	1447	301	20.8

Table 7. Results Intra-assay and Interassay Precision Tests Performed at Lab B. Values were calculated from Toxoplasma IgM EIA Index and IU/ml Values.

	INTRA-ASSAY						INTERASSAY					
SAMPLE	MEAN INDEX	S.D	C.V%	MEAN IU/ml	S.D.	C.V%	MEAN. INDEX	S.D.	C.V%	MEAN IU/ml	S.D	C.V%
Pos Control	2.2	0.231	10.3	942	188	20.0	2.3	0.187	8.1	1008	158	15.7
Neg Control	0	0	NA	0	0	NA	0	0.044	NA	7	10	NA
1	0.2	0.058	NA	86	16	NA	0.2	0.122	NA	87	42	NA
2	0	0	NA	8	8	NA	0	0.044	NA	11	15	NA
3	1.2	0.100	8.3	416	48	11.5	1.5	0.264	17.8	521	102	19.5
4	1.8	0.153	8.6	624	45	7.3	1.8	0.112	6.3	624	49	7.8
5	1.8	0.208	11.4	646	65	10.1	2.0	0.173	8.7	734	83	11.3
6	1.2	0.153	13.1	417	57	13.6	1.5	0.295	19.4	536	100	18.6
7	1.6	0.173	10.8	562	52	9.3	1.8	0.201	11.4	621	70	11.2
8	2.6	0.153	5.8	1293	121	9.3	2.8	0.245	8.7	1445	214	14.8

Table 8. Results Intra-assay and Interassay Precision Tests Performed at Lab C. Values were calculated from Toxoplasma IgM EIA Index and IU/ml Values.

	INTRA-ASSAY						INTERASSAY					
SAMPLE	MEAN INDEX	S.D	C.V%	MEAN IU/ml	S.D.	C.V%	MEAN. INDEX	S.D.	C.V%	MEAN IU/ml	S.D	C.V%
Pos. Control	2.5	0.115	4.6	1263	125	9.9	2.6	0.132	5.2	1295	120	9.2
Neg. Control	0.4	0.058	NA	138	9	NA	0.3	0.053	NA	123	15	NA
1	0.4	0.058	NA	154	18	NA	0.3	0.050	NA	139	17	NA
2	0.2	0	NA	88	3	NA	0.2	0.033	NA	81	7	NA
3	1.8	0.200	11.1	645	123	19.0	1.6	0.132	11.5	592	81	13.6

Subject/Title: ASI TOXOPLASMA IgM EIA TEST		Doc#: 810096AM
Effective Date: 02/18/16	Supersedes Revision/Date: Original	Revision: 02/18/16

4	2.0	0.058	2.8	830	63	7.6	1.9	0.136	7.2	721	97	13.5
5	2.2	0.115	5.3	935	105	11.2	2.1	0.112	5.4	847	95	11.2
6	2.0	0.058	2.9	757	38	5.0	1.8	0.112	6.1	679	63	9.2
7	2.3	0.058	2.5	1084	78	7.2	2.2	0.158	7.1	995	142	14.3
8	2.9	0.100	3.4	1590	65	4.1	3.1	0.206	6.7	1758	176	10.0

Clinical Sensitivity and Specificity

The serological diagnosis of toxoplasmosis should be based on both IgG and IgM assay results. To demonstrate this, serum specimens from18patients tentatively diagnosed with toxoplasma infections and confirmed by single-point commercial anti-toxoplasma gondii testing, and 32 patients tentatively diagnosed with cytomegalovirus infections, were assayed by the Toxoplasma IgG and IgM EIA tests. The results of these tests are presented below in Table 9.

Table 9. Results Toxoplasma IgM EIA and Toxoplasma IgG EIA Assays of 18 Serum Specimens Obtained From Patients Tentatively Diagnosed With Toxoplasma Infections, and 32 Specimens From Patients Tentatively Diagnosed With Cytomegalovirus Infections.

		Type of Infection	
Anti-T. gondii IgM Result	Anti-T. gondii IgG Result	Toxoplasma (18 Specimens)	Other (32 Specimens)
Negative	Negative	0	28
Negative	Positive	3	2
Negative	Equivocal	0	0
Equivocal	Negative	0	1
Equivocal	Positive	1	0
Equivocal	Equivocal	0	0
Positive	Negative	3	1
Positive	Positive	11	0
Positive	Equivocal	0	0

16.0 REFERENCES

1. Atlman, K.A., Toxoplasmosis, N.Y.State J. Med., 68(16):2151-2157, 1968.
2. Remington, J.S., Toxoplasmosis in the Adult, Bull. N.Y. Acad. Med., 50 (2): 211-227, 1974.
3. McLeod, R. and Remington, J.S., in Harrison's Principles of Internal Medicine (Isselbacher et al eds), McGraw-Hill Book Co., 879-885, 1980.
4. Frenkel, J.K., Toxoplasma in and Around Us, Bioscience, 23: 343-352, 1973.
5. Feldman, H.A., Epidemiology of Toxoplasma Infections,Epid. Rev., 4:204-213, 1982.
6. Walls, K.W. Serodiagnosis of Toxoplasmosis. Lab. Management 16: 26-31, 1973.
7. Remington, J.S. Toxoplasmosis, in *Obstetric and Prenatal Infections* (Charles, D. and Finland, M., eds). Lea Febiger, Philadelphia, pages 27-74, 1973.
8. *A Procedural Guide to the Performance of the Serology of Toxoplasmosis.* Centers for Disease Control, Atlanta, 1976.
9. Lin, T.M., Halbert, S.P., and O'Connor, G.R. Standardized Quantitative Enzyme-Linked Immunoassay for Antibodies to *Toxoplasma gondii.,* J.Clin. Microbiol. 11: 675-681, 1980.
10. Walls, K.W., Serodiagnosis of Toxoplasmosis, Laboratory Management 26-31, January,1978.
11. Duncan, R.J.S., The Use of ELISA for Rapid Viral Diagnosis: Antibody Detection. In *ELISA and Other Solid Phase Immunoassays*, D.M. Kemeny and S.J. Challacombe, eds. John Wiley & Sons, Ltd., Chichester.
12. Schaffer, J.G. and McQuay, R.M., Medical Protozoology, in Clinical Diagnosis by Laboratory Methods (Davidson, I. and Henry, J.B.eds.), W.B. Saunders Company, Philadelphia, 1969.
13. Lin, T.M. et al, J. Clin. Microbiol. 23: 77-82, 1982.
14. Franco, L. et al., Reverse enzyme-immunoassay for detection of specific anti-toxoplasma immunoglobulin M antibodies. J. Clin. Microbiol. 13: 859-864, 1981.
15. Welch, P.C., J. Infect. Dis., 142: 256-264, 1980.
16. Gardner, M.J. and Altman, D.G., Confidence Intervals Rather Than Hypothesis Testing. Brit. Med. J., 292: 746-750,1986.

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