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**TOXO IgM IFA TEST SYSTEM**  
**Catalog #431508-M**

**INTENDED USE**

The Diagnostic Automation, Inc. Toxo IgM IFA test system is an indirect fluorescent antibody assay designed for the presumptive qualitative detection of IgM antibodies to *Toxoplasma gondii* in human serum and for the presumptive diagnosis of acute, recent, or reactive *Toxoplasma gondii* infection. To adequately assess the patient's serological status, testing must be performed in conjunction with an anti-*Toxoplasma gondii* IgG antibody assay. This product is not FDA cleared (approved) for use in testing (*i.e.*, screening) blood or plasma donors. This assay's performance has not been established for screening of prenatal women or newborns.

**SIGNIFICANCE AND BACKGROUND**

*T. gondii* is an obligate intracellular protozoan parasite with a worldwide distribution (1,2). Although cats are the definitive host, the organism can infect almost all mammals and birds. Serological data indicates that approximately 30% of the population of most industrialized nations is chronically infected with the organism, although the prevalence varies among different populations (3).

Toxoplasma exists in three forms: trophozoite, cysts, and oocysts (1,2). 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 and may contain up to several thousand organisms. Oocysts develop in the intestinal epithelial cells of cats and are not found in other hosts. Oocysts are excreted in the feces of cats and mature after a few days.

Infection of man and other animals occurs after ingestion of either cysts in raw or undercooked meat, or mature oocysts in material contaminated with cat feces. Once ingested, the parasites are liberated from cysts or oocysts by digestive enzymes and invade the intestinal mucosa. The parasites multiply locally and are then transported to other organs forming tissue cysts which persist for the life of the host. Cysts are found predominantly in brain, heart, and skeletal muscle.

Infection with *T. gondii* is asymptomatic in the majority (80-90%) of cases (4). The most common clinical manifestation of acute toxoplasmosis in the adult is asymptomatic lymphadenopathy involving single or multiple nodes. Lymphadenopathy may be

accompanied by fever, malaise, and atypical lymphocytosis symptoms which mimic infectious mononucleosis. Very rarely will more serious complications, such as encephalitis, myocarditis or pneumonitis, be seen in the normal host (1).

Although the normal host usually suffers no ill effects from infection with *T. gondii*, infection in an immunocompromised host is often fatal (5). Immunocompromised patients may develop severe disseminated toxoplasmosis or toxoplasmic encephalitis, or both. Toxoplasma is a common opportunistic infection of the central nervous system in patients with acquired immunodeficiency syndrome (AIDS) (6). Serologic evidence indicates that toxoplasmic encephalitis in AIDS patients results from reactivation of latent infections. Approximately 30% of AIDS patients who are toxoplasma antibody positive will develop toxoplasmic encephalitis (7).

When a seronegative women becomes infected with *T. gondii* during pregnancy, the organism is often transmitted across the placenta to the fetus (1,8). The severity of infection in the fetus varies with the trimester during which the infection was acquired. Infection during the disease in the neonate. Infection acquired later during pregnancy is usually asymptomatic in the neonate, and may not be recognized (8).

Approximately 75% of congenitally infected newborns are symptomatic. However, nearly all children born with subclinical toxoplasmosis will develop adverse ocular or neurologic sequelae later in life. Approximately 80-85% develop chorioretinitis and some may also experience blindness or mental retardation.

A variety of serologic tests for antibodies to *T. gondii* have been used as an aid in diagnosis of acute infection and to assess previous exposure to the organism. The more widely used tests include the Sabin-Feldman dye test, direct agglutination, indirect hemagglutination, latex agglutination, indirect immunofluorescence, and ELISA (9). Serologic procedures that measure IgM class antibodies to *T. gondii* include indirect immunofluorescence, immunosorbent agglutination, and ELISA (4,9,10,11).

High affinity IgG antibodies to *T. gondii*, if present in a sample, may interfere with the detection of IgM specific antibody. High affinity IgG antibody may preferentially bind to *T. gondii* antigen leading to false negative IgM results (12); also, rheumatoid factor, if present along with antigen-specific IgG, may bind to the IgG causing false positive IgM results (13).

Both of the above problems can be eliminated by removing IgG from the sample before testing for IgM (14,15,16). Several different methods of separating IgG have been used. These include gel filtration (14), absorption with protein A (9), ion exchange chromatography (15), precipitation of IgG with anti-human IgG serum (16). or the use of **ZORBA®** IgG Removal Reagent .

#### **PRINCIPLE OF THE IFA ASSAY**

The Diagnostic Automation, Inc. *T. gondii* IgM IFA test system is designed to detect IgM class antibodies to *T. gondii* antigen. The test system employs *T. gondii* substrate and

fluorescein labeled anti-human IgM ( $\mu$  chain specific). The test procedure involves three incubation steps:

1. Test sera are first treated to remove IgG and rheumatoid factor.
2. Test sera, properly treated and diluted, are added to the wells and incubated. Antigen specific IgM antibody will bind to *T. gondii* substrate immobilized on the slide. The slides are washed to remove unbound antibody and other serum components.
3. Fluorescein labeled anti-human IgM conjugate is added to the wells and the slides are incubated. The conjugate will react with the antigen specific IgM antibodies bound to the slides in step 2. The slides are washed to remove unbound conjugate. The slides are then mounted with a coverslip and read under a fluorescence microscope.

## **KIT COMPONENTS**

### **Reactive Reagents:**

1. *T. gondii* antigen slides: Fifteen 8-well substrate slides containing *T. gondii* organisms (strain RH).
2. Goat anti-human IgM ( $\mu$  chain specific) labeled with fluorescein. Contains 1.0% bovine albumin and Evans blue counterstain. One 3ml vial, lyophilized.
3. *T. gondii* human positive control serum: One 0.5ml vial, lyophilized.
4. *T. gondii* human negative control serum: One 0.5ml vial, lyophilized.

### **Non-reactive components:**

1. Phosphate-Buffered-Saline (PBS): Five packets. Sufficient to prepare 5 liters of PBS (0.1M phosphate, 0.15M NaCl, pH 7.6).
2. Mounting Fluid: One 3.0ml vial of phosphate-buffered-glycerol, pH 9.0..

**CAUTION:** All reactive reagents, as well as buffered glycerol contain a preservative which may be toxic if ingested (thimerosal, mercury derivative 0.04% w/v).

## **PRECAUTIONS**

1. For *in vitro* diagnostic use.
2. Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

## **WARNING - POTENTIAL BIOHAZARDOUS MATERIAL**

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (23).

3. Each working reagent has been optimized to identify *T. gondii* IgM antibody.
4. Dilution or adulteration of these reagents may result in loss of sensitivity.
5. Reagents from other sources or manufacturers should not be used.

6. The concentration of anti-*Toxoplasma gondii* IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
7. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
8. Avoid microbial contamination of reagents. Incorrect results may occur.
9. Incubation times or temperatures other than those specified may give erroneous results.
10. All reagents should be brought to room temperature (20-25°C) and mixed well before use.
11. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
12. Evans blue dye is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
13. Although the slides have been inactivated, they should be handled as if capable of transmitting infection.
14. Assay **reproducibility** has not been established.

#### **ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED**

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Small test tubes, 13 x 100 mm or comparable.
3. Test tube racks.
4. Staining dish - A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slips: 24 x 60mm, thickness No. 1.
6. Distilled water.
7. One liter volumetric flask.
8. Clean wash bottle
9. Moist 37°C incubation chamber.
10. IgG removal system (See Limitations, #2).
11. Properly equipped fluorescent microscope assembly. Fluorescein is excited at 490 nm and emits at 520nm.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies.

<b>TRANSMITTED LIGHT</b>		
Light Source: Mercury vapor 200 W or 50 W		
Excitation filter	Barrier filter	Red Suppression filter
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten - Halogen 100 W		
KP490	K510 or K530	BG38

<b>INCIDENT LIGHT</b>			
Light Source: Mercury vapor 200, 100, 50 W			
Excitation filter	Dichroic Mirror	Barrier filter	Red Suppression filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten - Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

## **SPECIMEN COLLECTION**

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be employed in this assay (17,18). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

## **STORAGE CONDITIONS**

- *T. gondii* Substrate Slides: -20°C or lower.
- Goat anti-human IgM labeled with FITC: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Positive and negative human *T. gondii* control sera: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Phosphate-buffered-saline: Store at 2-25°C. Store reconstituted buffer at 2-8°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
- Buffered glycerol (mounting media): Store at 2-8°C.

## **NOTE:**

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.

## **PROCEDURE**

### **Preparation of Reagents:**

1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. *T. gondii* human positive and negative control sera. Reconstitute with 0.5ml of distilled water.
3. Goat anti-human IgM FITC-labeled conjugate. Reconstitute with 3.0ml of distilled water. Alternately, aliquot in 0.5ml amounts and store at -20°C or lower in small tubes for up to six months.

**NOTE:** Reconstitute reagents gently but thoroughly. If lyophilized reagents show evidence of rehydration prior to reconstituting, do not use. If reagents become cloudy, bacterial contamination should be suspected, and reagents should not be used.

## **TEST PROCEDURE**

1. Remove substrate slides from freezer and allow them to reach room temperature (20-25°C). Tear open the protective envelope and remove slides containing the *T. gondii* substrate slides. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE, THIS COULD DESTROY THE CELL SHEET ON THE SLIDE.**

2. Pretreat the test sera to remove IgG. Precipitation with anti-human IgG is recommended because this procedure is effective in removing all subclasses of human IgG and is less cumbersome to perform than other methods.
3. After the pretreatment step, test sera should be at a 1:10 screening dilution. The prediluted positive and negative serum controls, and a buffer control should be run each time the test is performed.
4. Identify each well with the appropriate patient sera and controls.
5. Spread 20 $\mu$ l of test and control sera over each appropriately labeled well being careful not to disturb the substrate cells with pipette tip.
6. Incubate slides in a moist chamber at 37°C for one hour  $\pm$  5 minutes.
7. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. **DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS.**
8. Place slides in a staining dish and wash in PBS, for two, 5 minute intervals, with a change of PBS.
9. Remove slides from PBS solution. Rinse briefly with distilled or deionized water and air dry slides. Do not disturb substrate organisms.
10. Place slides in a moist chamber and add 20 $\mu$ l conjugate to each well.
11. Incubate slides in a moist chamber at 37°C for 30 minutes  $\pm$  5 minutes. **DO NOT ALLOW WELLS TO DRY.**
12. Repeat steps 7, 8, and 9.
13. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Avoid entrapment of air bubbles. Slides should be examined immediately at a total magnification of 400X.

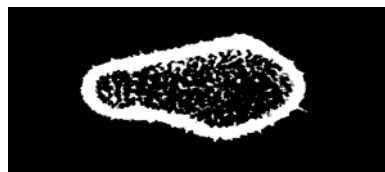
### **QUALITY CONTROL**

1. Positive, negative, and buffer controls should be run with each assay. The buffer control is included to test for nonspecific interaction between the conjugate and the substrate and therefore should be negative.
2. It is recommended that one read the positive and negative controls before evaluating test results. This will assist in establishing the positive and negative references required to interpret the test samples. If the controls do not appear as described, results are invalid and patient results may not be reported (See Figure 1).
3. The negative control is characterized by the absence of staining along the periphery of the substrate organism. The organisms will appear reddish with no yellow-green fluorescence. Use the reaction of the negative control serum as a guide for the interpretation of patient results.
4. The positive control is characterized by confluent 1+ to 4+ apple-green fluorescent staining along the periphery of the substrate organisms. A 1+ reaction is one that shows weak but distinct apple-green fluorescence and represents the endpoint reaction in a titration. A 4+ reaction is one that shows very strong apple-green staining at the periphery of the substrate organisms.
5. The intensity of the observed fluorescence may vary with the microscope and filter system used.

**Figure 1**  
Pictorial Representation of Reactive and Non-Reactive Staining Patterns

**POSITIVE**

Reaction is positive when yellow-green fluorescence extends around the entire periphery of the organism. This reaction may be intense enough (in low dilutions of strong positive sera) to mask all internal red counterstain. In higher dilutions, the peripheral staining will become a thin peripheral halo around an internal red fluorescence.

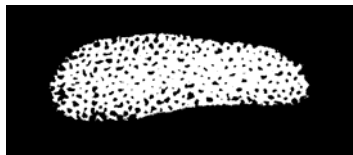


**Yellow-green periphery**

**Yellow- green organism**



Reaction is negative when organisms appear reddish with no yellow-green fluorescence around the periphery. Reaction is also considered negative when only one end of the organism fluoresces bright yellow-green with no extension of yellow-green around the other end (polar staining). Non-confluent fluorescence (beaded) is considered to be negative for specific antibody.



**Negative**



**Polar Staining**



**Beaded Staining**

## INTERPRETATION OF RESULTS

### TITER CLINICAL SIGNIFICANCE

<1:10

**NEGATIVE:** No detectable IgM antibody to *T. gondii*. A negative result indicates no primary or recent infection with *T. gondii* and implies that such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken within 7 days to look for the presence of *T. gondii* specific IgM. If the second specimen is positive, a primary or recent infection with *T. gondii* is indicated. Alternatively, additional samples may be drawn 2-8 weeks later and analyzed for the presence of Toxo-specific IgG. The appearance of Toxo-specific IgG in a patient previously shown to be negative for Toxo IgG indicates that a primary infection has occurred. A polar staining reaction is considered negative for specific antibody to *T. gondii*. Finally, it should be noted that a non-confluent or beaded fluorescent pattern is also considered negative for specific antibody to *T. gondii* (see Figure 1).

### TITER

>1:10

**POSITIVE:** Detectable IgM antibody to *T. gondii*. This indicates a primary or recent infection with *T. gondii*. Such individuals are presumed to be at risk of transmitting *T. gondii* infection (see Figure 1).

Anti- <i>T. gondii</i> IgM Result	Anti- <i>T. gondii</i> IgG Result	Report/Interpretation
Negative	Negative	It is presumed the patient has not been infected with and is not undergoing an acute infection with <i>Toxoplasma gondii</i> . 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 <i>Toxoplasma gondii</i> infection. It appears the patient has been previously infected with <i>Toxoplasma gondii</i> . 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 <i>Toxoplasma gondii</i> . Determining whether the patient has been previously infected with <i>Toxoplasma gondii</i> is not possible.
Equivocal	Negative	Obtain a new specimen for determination of IgM antibodies to <i>Toxoplasma gondii</i> . It cannot be determined if the patient is undergoing an acute <i>Toxoplasma gondii</i> infection. It appears the patient has not been previously infected with <i>Toxoplasma gondii</i> . 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 <i>Toxoplasma gondii</i> . It cannot be determined if the patient is undergoing or has undergone an acute <i>Toxoplasma gondii</i> infection. It appears the patient has been previously infected with <i>Toxoplasma gondii</i> . 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.

<b>Anti-<i>T. gondii</i> IgM Result</b>	<b>Anti-<i>T. gondii</i> IgG Result</b>	<b>Report/Interpretation</b>
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 <i>Toxoplasma gondii</i> . 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 <i>Toxoplasma gondii</i> . Since the IgG antibodies to <i>Toxoplasma gondii</i> 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- <i>Toxoplasma gondii</i> 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 <i>Toxoplasma gondii</i> . Obtain a new specimen for further testing. Since the IgG antibodies to <i>Toxoplasma gondii</i> are positive, it appears the patient may be acutely infected with <i>Toxoplasma gondii</i> . The new specimen should be repeated with a different anti- <i>Toxoplasma gondii</i> IgM assay. If the new

		specimen result is still positive for IgM and IgG antibodies to <i>Toxoplasma gondii</i> , 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 <i>Toxoplasma gondii</i> . Obtain a new specimen for further testing. Determining whether the patient has been previously infected with <i>Toxoplasma gondii</i> 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- <i>Toxoplasma gondii</i> IgM assay. If the new specimen result is still positive for IgM and the IgG is positive/negative/equivocal for antibodies to <i>Toxoplasma gondii</i> the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

## LIMITATIONS OF THE ASSAY

1. Substitution of other reagents for components of this kit is to be avoided. Since the components of this kit have been tested for maximum efficiency, Diagnostic Automation Inc is not responsible for test performance if reagent substitution occurs.
2. IgG antibodies, if present in the sample, may interfere with determination of IgM titers to the organism. High affinity IgG antibodies may preferentially bind to antigenic determinants leading to false negative IgM titers. Also, IgM rheumatoid factor may bind to the antigen specific IgG leading to false positive IgM titers. Both of these problems can be eliminated by removing IgG from the samples before testing for IgM (14,15,16). Several different methods of separating IgG have been used. These include gel filtration (14), absorption with protein A (9), ion exchange chromatography (15), precipitation of IgG with anti-human IgG serum (16), or the use of ZORBA-IgG Removal Reagent .
3. Results of the Diagnostic Automation Inc Toxo IgM IFA are not by themselves diagnostic and should be interpreted in light of the patients clinical condition and the results of other diagnostic procedures.
4. Samples taken too early during the course of a primary infection with *T. gondii* may not contain detectable levels of IgM-specific antibody (4). In some patients, IgM specific antibody results may revert to negative levels within three weeks after infection with *T. gondii* (1). Measurement of *T. gondii*-specific IgG antibodies may also be of some value in the serological assessment of these patients.
5. *T. gondii*-specific IgM antibody may not be demonstrable in patients who are immunocompromised and in some patients with congenital toxoplasmosis (2).
6. Naturally occurring *T. gondii*-specific IgM antibodies, with or without the occurrence of IgG antibodies, have been reported (19,20). Neither the stimulus nor the significance of naturally occurring IgM antibodies directed against *T. gondii* is understood at this time.
7. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr Virus and give false positive results on the Toxo IgM IFA test system.
8. *T. gondii*-specific IgG antibody may compete with specific IgM for antibody binding and cause false negative results. Rheumatoid factor (IgM), if present with *T. gondii*-specific IgG, will cause false positive results. The absorbent incubation step will functionally remove greater than 99% of IgG from the test specimen and significantly reduce the possibility of false positive or negative results.
9. False positive anti-Toxoplasma results have been reported for patients having autoimmune disease (21).
10. The performance of the Diagnostic Automationinc Toxo IgM IFA has not been validated using neonatal samples.
11. A negative result for Toxo IgM does not preclude the possibility of an acute infection in the immunocompromised patient. *T. gondii*-specific IgG antibodies are generally low and *T. gondii*-specific IgM antibodies may be undetectable in patients who are immunocompromised (22).
12. Due to the apparent low prevalence of anti-*Toxoplasma gondii* IgM in the United States the performance characteristics cited below may not be representative of the population at each users laboratory.

13. With very low prevalence analytes, such as anti-*Toxoplasma gondii* IgM there is the increased possibility that a positive result is truly a false positive, reducing the assay's positive predictive value (24-27).

### **EXPECTED VALUES**

*T. gondii*-specific IgM antibodies rise sharply just before or shortly after onset of symptoms, and reach peak titers within one month (1,4). *T. gondii*-specific IgM falls to low levels in most patients within 4 to 6 months (4). In some patients, IgM-specific antibodies may be detectable for 8 months to one year (1,11).

In a study conducted by Diagnostic Automation Inc., 108 samples were tested for Toxo IgM using the Diagnostic Automation Inc. ToxoIgM IFA. These samples (from Northeastern U.S.) were sent to a reference laboratory for routine Toxo serological analysis. Five of the 108 samples (4.6%) were reactive for Toxo IgM. As with any serological method, the expected values are highly dependent upon the population type being tested. Each laboratory should establish their own expected values based upon the population type tested.

As part of the clinical study, a group of 130 asymptomatic, 'normal' specimens were tested. A summary of this testing is shown in Table 3.

### **PERFORMANCE CHARACTERISTICS**

#### **Comparative Study:**

The Diagnostic Automation Inc. Toxo-IgM IFA test system was compared to a commercially available IgM Capture ELISA for the detection of IgM antibodies to *Toxoplasma gondii*. In this study, a total of 156 serum samples were evaluated. Twenty-six Toxo IgM positive samples were obtained from a Toxoplasma Reference lab, and 130 samples were from normal plasma donors from Southeastern United States. For all IgM IFA testing, IgG was removed by using ZORBA® IgG Removal Reagent. The presence of *T. gondii*-specific IgM in the samples from the reference laboratory was supported by the following information:

Positive by the Sabin-Feldman dye test, positive by reference laboratory Toxo IgM capture ELISA, and clinical diagnosis. Discrepant samples were evaluated using a commercial Toxoplasma IgM IFA test system. The results of these studies are summarized below:

**Table 1**  
**Diagnostic Automation Inc. Toxo IgM IFA**  
**Initial Comparison to a Commercial Toxo IgM Capture ELISA**

Relative Sensitivity: 92.3% (24/26)  
 Relative Specificity: 100% (112/112)  
 Percent Agreement: 98.6% (136/138)

**Table 2**  
**Toxoplasma Reference Laboratory Samples**  
**(n=26)**

		Diagnostic Automation Inc. Toxo IgM IFA			
		+	-	±	Totals
Commercial Toxo IgM ELISA (Capture)	+	24	2 <sup>a</sup>	0	26
	-	0	0	0	0
	±	0	0	0	0
					26

Samples that were equivocal on the IgM capture ELISA test system were excluded from the calculations.

Relative Sensitivity = 92.3% (24/26)

<sup>a</sup> For discrepant results, see Table 4.

**Table 3**

**Normal Plasma Donor Samples (n=130)**

		Diagnostic Automation Inc. Toxo IgM IFA			
		+	-	±	Totals
Commercial Toxo IgM ELISA (Capture)	+	0	11 <sup>a</sup>	0	11
	-	0	112	0	112
	±	0	7 <sup>b</sup>	0	7
					130

Relative Specificity = 100% (112/112)

<sup>a</sup> For discrepant results, see Table 4

<sup>b</sup> Equivocal results were excluded from calculations

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**NOTE:** Please be advised that 'relative' refers to the comparison of this 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

<b>Table 4</b>			
<b>Analysis of Discrepant Results by a Commercial IFA</b>			
<b>SAMPLED ID</b>	<b>DAI TOXO IgM IFA</b>	<b>COMMERCIAL TOXO IgM IFA</b>	<b>COMMERCIAL* TOXO IgM IFA</b>
120	-	+	-
121	-	+	-
122	-	+	-
123	-	+	-
124	-	+	-
125	-	+	-
126	-	+	-
127	-	+	-
128	-	+	-
129	-	+	-
130	-	+	-
132	-	+	+
156	-	+	+

\* Samples 120-130 exhibited polar staining which is not indicative of a specific positive reaction. These samples did not display a polar staining reaction on the DAI Toxo IgM IFA.

### **Comparison After Resolution of Discrepant Results**

Relative Sensitivity: 24/26 = 92.3%  
 Relative Specificity: 123/123 = 100%  
 Percent Agreement: 147/149 = 98.7%

**Cross Reactivity:**

Studies were performed to assess interference in the test procedure by rheumatoid factor (RF), EBV-IgM, CMV-IgM, Rubella IgM, and antibodies to nuclear antigens (ANA).

Nine samples containing EBV-IgM antibodies (IFA titer range = 1:10 to 1:5120), and 33 samples positive for RF by latex agglutination (titer range = 1:20 to 1:640) were negative when tested with the Diagnostic Automation Inc. Toxo IgM IFA. One sample, strongly positive for Toxo IgG and rheumatoid factor (1:160) produced a low to mid-positive IgM result when tested with Diagnostic Automation Inc. Toxo IgM IFA prior to IgG absorption. Following IgG absorption, the Toxo IgM IFA result was clearly negative. Two samples positive for CMV-IgM antibody by Diagnostic Automation Inc. ELISA, and four samples positive for Rubella-IgM antibody by Diagnostic Automation Inc. ELISA were tested on the Diagnostic Automation Inc. Toxo IgM IFA. All CMV samples, and three of the four Rubella samples were clearly negative. One Rubella IgM positive sample yielded a very weak ( $\pm$ ) beaded fluorescent pattern.

Finally, four samples exhibiting antibodies to nuclear antigens were tested on the Diagnostic Automation Inc. Toxo IgM IFA. All samples were clearly negative for Toxo IgM, yet two samples did display a very weak non-specific fluorescent pattern.

The results of the cross-reactivity investigation show that there exists little or no cross-reactivity with Diagnostic Automation Inc. Toxo IgM IFA.

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