T. gondii IgM Test System

REF FA8001M

IVD

Rx Only

INTENDED USE

The ZEUS IFA *Toxoplasma gondii* IgM Test System is an indirect fluorescent antibody assay designed for the presumptive qualitative detection of IgM antibodies to *T. gondii* in human serum and for the presumptive diagnosis of acute, recent, or reactive *T. gondii* infection. To adequately assess the patient's serological status; testing must be performed in conjunction with an anti-*T. gondii* IgG antibody assay. This product is not FDA cleared (approved) for use in testing (*i.e.*, screening) blood or plasma donors. Performance characteristics have not been established for screening sera from 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 woman 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 first trimester may lead to spontaneous abortion, stillbirth, or overt 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 - 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 - 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 (ZEUS Product #: FA003G).

PRINCIPLE OF THE ASSAY

The ZEUS IFA *T. gondii* IgM Test System is designed to detect IgM class antibodies to the *T. gondii* antigen. The assay employs *T. gondii* organisms and fluorescein labeled anti-human IgM (µ chain specific). The assay procedure involves three incubation steps:

1. Step one; test sera are first treated to remove IgG and rheumatoid factor.
2. Step two; 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. Step three; 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.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •		1. *Toxoplasma gondii* Antigen Substrate Slides: Ten, 10-well Slides containing *T. gondii* organisms (strain RH). Also includes a desiccant pouch.
CONJ		2. Conjugate: Anti-human IgM (µ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): Will produce positive apple-green staining around the periphery of the organisms. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL	-	4. Negative Control (Human Serum): Will produce no detectable staining of the organisms. One, 0.5mL, green-capped, vial. Ready to use.
DIL	SPE	5. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
BUF	PBS	6. Phosphate-buffered-saline (PBS): pH 7.6 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.

MNTMED	7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA8009S), and PBS (Product #: 0008LTS).**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety 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 Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate.
7. The SAVe Diluent®, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Assay reproducibility has not been established.
23. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).
12. Incubator: 35 - 37°C.
13. IgG Removal System (see Limitations of the Assay).

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

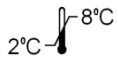
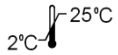
Transmitted Light		
Light Source: Mercury Vapor 200W or 50W		
Excitation Filter	Barrier Filter	Red Suppression Filter
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

Incident Light			
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

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (17, 18). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (28).

STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted**.
3. Diluting Patient Sera:
   a. It is recommended that test sera are pre-treated 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. After the pretreatment step, test sera should be at a 1:10 screening dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS).
   b. If patient samples are to be titrated to endpoint, one should pre-treat the serum to remove IgG and then make any subsequent dilutions with SAVe Diluent or PBS**. NOTE: The SAVe Diluent® will undergo a color change confirming the combination of specimen with Diluent.**
   c. As an option, users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
4. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
5. Incubate Slides at 35 - 37°C for 60 ± 5 minutes.
6. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
7. Wash Slides for two, 5 minute intervals, changing PBS between washes.
8. Remove Slides from PBS. Rinse Slides briefly with deionized or distilled water and air dry Slides. Do not disturb the organisms in the wells.
9. Add 20µL of Conjugate to each well. Incubate Slides at 35 - 37°C for 30 ± 5 minutes.
10. Repeat steps 6 through 8.
11. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

QUALITY CONTROL

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
   a. Negative Control - characterized by the absence of staining along the periphery of the substrate organism. The organisms will appear reddish, or dull green, with no yellow-green fluorescence. Use the reaction of the negative control serum as a guide for the interpretation of patient results.
   b. Positive Control - characterized by confluent 1+ to 4+ apple-green fluorescent staining along the periphery of the 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 organisms.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The intensity of the observed fluorescence may vary with the microscope and filter system used.

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 *T. gondii* 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*.
>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.

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 *T. 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 *T. gondii* infection. It appears the patient has been previously infected with *T. 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 *T. gondii*. Determining whether the patient has been previously infected with *T. gondii* is not possible.
Equivocal	Negative	Obtain a new specimen for determination of IgM antibodies to *T. gondii*. It cannot be determined if the patient is undergoing an acute *T. gondii* infection. It appears the patient has not been previously infected with *T. 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 *T. gondii*. It cannot be determined if the patient is undergoing or has undergone an acute *T. gondii* infection. It appears the patient has been previously infected with *T. 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 *T. 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 *T. gondii*. Since the IgG antibodies to *T. 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-*T. 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 *T. gondii*. Obtain a new specimen for further testing. Since the IgG antibodies to *T. gondii* are positive, it appears the patient may be acutely infected with *T. gondii*. The new specimen should be repeated with a different anti-*T. gondii* IgM assay. If the new specimen result is still positive for IgM and IgG antibodies to *T. 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 *T. gondii*. Obtain a new specimen for further testing. Determining whether the patient has been previously infected with *T. 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-*T. gondii* IgM assay. If the new specimen result is still positive for IgM and the IgG is positive/negative/equivocal for antibodies to *T. gondii* the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

LIMITATIONS OF THE ASSAY

1. 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 - 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.
2. Results of the ZEUS IFA *T. gondii* IgM Test System are not by themselves diagnostic and should be interpreted in light of the patients clinical condition and the results of other diagnostic procedures.
3. 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.
4. T. *gondii*-specific IgM antibody may not be demonstrable in patients who are immunocompromised and in some patients with congenital toxoplasmosis (2).
5. 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.
6. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr Virus and give false positive results on the ZEUS IFA *T. gondii* IgM Test System.
7. 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.
8. False positive anti-*T. gondii* results have been reported for patients having autoimmune disease (21).
9. The performance of the ZEUS IFA *T. gondii* IgM Test System has not been validated using neonatal samples.
10. 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).
11. Due to the apparent low prevalence of anti-*T. gondii* IgM in the United States the performance characteristics sited below may not be representative of the population at each users laboratory.
12. With very low prevalence analytes, such as anti-*T. 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 RESULTS

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 ZEUS Scientific, 108 samples were tested for *T. gondii* IgM using the ZEUS IFA *T. gondii* IgM Test System. 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 *T. gondii* 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

1. **Comparative Study:**

The ZEUS IFA *T. gondii* IgM Test System was compared to a commercially available IgM Capture ELISA for the detection of IgM antibodies to *T. gondii*. In this study, a total of 156 serum samples were evaluated. Twenty-six *T. gondii* 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 *T. gondii* IgM IFA test system. The results of these studies are summarized below:

Table 1: ZEUS IFA *T. gondii* IgM Test System 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)

Samples that were equivocal on the IgM capture ELISA Test System were excluded from the calculations.

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

		ZEUS IFA *T. gondii* IgM Test System			
		Positive	**Negative**	**Equivocal**	**Totals**
Commercial Toxo IgM ELISA (Capture)	**Positive**	24	2[a]	0	26
	Negative	0	0	0	0
	Equivocal	0	0	0	0
	Totals	24	2[a]	0	26

Relative Sensitivity = 92.3% (24/26) [a]For discrepant results, see Table 4.

Table 3: Normal Plasma Donor Samples (n=130)

		ZEUS IFA *T. gondii* IgM Test System			
		Positive	**Negative**	**Equivocal**	**Totals**
Commercial Toxo IgM ELISA (Capture)	**Positive**	0	11[a]	0	11
	Negative	0	112	0	112
	Equivocal	0	7[b]	0	7
	Totals	0	130	0	130

Relative Specificity = 100% (112/112) [a]For discrepant results, see Table 4 [b]Equivocal results were excluded from calculations

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.

Table 4: Analysis of Discrepant Results by a Commercial IFA

Sample ID	ZEUS IFA *T. gondii* IgM	Commercial Toxo IgM IFA	Commercial* Toxo IgM IFA
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 ZEUS IFA *T. gondii* IgM Test System.

Table 5: Comparision After Resolution of Discrepant Results

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

2. **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 ZEUS IFA *T. gondii* IgM Test System. One sample, strongly positive for T *T. gondii* IgG and Rheumatoid Factor (1:160) produced a low to mid-positive IgM result when tested with the ZEUS IFA *T. gondii* IgM Test System prior to IgG absorption. Following IgG absorption, the ZEUS IFA *T. gondii* IgM Test System result was clearly negative. Two samples positive for CMV-IgM antibody by ZEUS ELISA, and four samples positive for Rubella-IgM antibody by the ZEUS ELISA were tested on the ZEUS IFA *T. gondii* IgM Test System. All CMV samples, and three of the four Rubella samples were clearly negative. One Rubella IgM positive sample yielded a very weak (±) beaded fluorescent pattern. Finally, four samples exhibiting antibodies to nuclear antigens were tested on the ZEUS IFA *T. gondii* IgM Test System. All samples were clearly negative for Toxoplasma 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 the ZEUS IFA *T. gondii* IgM Test System.

REFERENCES

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