

INTENDED USE

Rx Only

The ZEUS ELISA *Toxoplasma gondii* IgM Test System is an enzyme-linked immunosorbent 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 screening blood or plasma donors. This assay's performance has not been established for screening of prenatal women or newborns.

SIGNIFICANCE AND BACKGROUND

Toxoplasma 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 chronic infection with the organism affects approximately 30% of the population of most industrialized nations, 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. Formation of tissue cysts occur 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 develop only in the intestinal epithelial cells of cats, and once excreted in the feces, mature within a few days.

Infection occurs in man and other animals after ingestion of cysts in raw or undercooked meat, or mature oocysts in material contaminated with cat feces. Ingestion liberates the parasites from the cysts or oocysts because of digestive enzymes. Once liberated the parasites invade the intestinal mucosa, multiply locally, and are then transported to other organs to form tissue cysts that persist for the life of the host. Research shows that cysts are predominant in the 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. Symptoms of lymphadenopathy include fever, malaise, and atypical lymphocytosis (symptoms that 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).

Infection of a seronegative woman with *T. gondii* during pregnancy often results in transmission of the organism across the placenta to the fetus (1, 8). Trimester of acquisition of the organism determines the severity of infection in the fetus. 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% of children develop chorioretinitis and some may experience blindness or mental retardation.

Varieties of serologic tests for antibodies to *T. gondii* may aid in the diagnosis of acute infection and 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 enzyme-linked immunosorbent assays (ELISA (9)). Antibodies of the IgM class appear during the first week of a primary infection with *T. gondii* and exist only transiently in most patients (1). Serologic procedures that measure IgM antibodies help identify patients with recently acquired infections (1, 2, and 4). Serologic procedures that measure IgG class antibodies to *T. gondii* include indirect fluorescence, immunosorbent agglutination, and ELISA (4, 9, 10, and 11). Engvall and Perlman (12, 13) first described the ELISA procedure. Since then, scientists have developed ELISA test systems for detection of a wide variety of different antigens and antibodies (14). ELISA may be a very specific, sensitive, and reliable method for detection of antibodies to *T. gondii* (9, 11). The ELISA procedure allows for an objective determination of antibody status using a single dilution of the test specimen and is suitable for screening large numbers of patient samples.

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 (14); also, rheumatoid factor, if present along with antigen-specific IgG, may bind to the IgG causing false positive IgM results (15). Remove IgG from the sample before testing for IgM to aid in the elimination of both of the above problems (16, 17, and 18). Scientists have used several different methods of separating IgG from the sample, including gel filtration (16), absorption with protein A (9), ion exchange chromatography (17), and precipitation of IgG with anti-human IgG serum (18).

PRINCIPLE OF THE ASSAY

The ZEUS ELISA *T. gondii* IgM Test System is designed to detect IgM class antibodies to *Toxoplasma gondii* in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with *T. gondii* antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG which precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen. During sample incubation, any antigen specific IgM antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

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: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and Sample Diluent.**

- | | | |
|-----------|----|--|
| PLATE | 1. | Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated <i>T. gondii</i> antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant. |
| CONJ | 2. | Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific). One, 15mL, white-capped bottle. Ready to use. |
| CONTROL + | 3. | Positive Control (Human Serum): One, 0.35mL, red-capped vial. |
| CAL | 4. | Calibrator (Human Serum): One, 0.5mL, blue-capped vial. |
| CONTROL - | 5. | Negative Control (Human Serum): One, 0.35mL, green-capped vial. |

DIL	SPE	6. Sample Diluent: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Purple Solution. Ready to use.
SOLN	TMB	7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H ₂ SO ₄ , 0.7M HCl. Ready to use.
WASHBUF	10X	9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

NOTES:

- The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer.
- Test System also contains a Component Label containing lot specific information inside the Test System box.

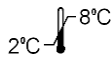
PRECAUTIONS

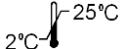
- For *In Vitro* diagnostic use.
- Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
- The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
- The Controls are **potentially biohazardous 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, handle these products 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 Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (25).
- 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 refrigerated temperature immediately after use.
- Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
- The Sample Diluent, Controls, and Calibrator 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
- The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
- The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
- The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
- Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
- Dilution or adulteration of these reagents may generate erroneous results.
- Do not use reagents from other sources or manufacturers.
- TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross contamination of reagents and/or samples could cause erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Avoid splashing or generation of aerosols.
- Do not expose reagents to strong light during storage or incubation.
- Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
- 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.
- Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
- Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
- Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
- 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.

MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
- Pipettes capable of accurately delivering 10 - 200µL.
- Multichannel pipette capable of accurately delivering 50 - 200µL.
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent

	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C
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SPECIMEN COLLECTION

- ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
- No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
- Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (20). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
- 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 a 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 (30).

ASSAY PROCEDURE

- Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
- Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

- Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
- To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
- Add 100µL of Sample Diluent to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
- Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
- Wash the microwell strips 5 times.
 - Manual Wash Procedure:**
 - Vigorously shake out the liquid from the wells.
 - Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
 - Repeat steps 1. and 2. for a total of 5 washes.
 - Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
 - Automated Wash Procedure:**
If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
- Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
- Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
- Wash the microwells by following the procedure as described in step 7.
- Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
- Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
- Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
- Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

- Dilute Serum 1:21.
- Add diluted sample to microwell - 100µL/well.
- Incubate 25 ± 5 minutes.
- Wash.
- Add Conjugate - 100µL/well.
- Incubate 25 ± 5 minutes.
- Wash.
- Add TMB - 100µL/well.
- Incubate 10 - 15 minutes.
- Add Stop Solution - 50µL/well - Mix.
- READ within 30 minutes.

QUALITY CONTROL

- Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included in each assay.
- Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
- The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250

Calibrator	≥0.300
Positive Control	≥0.500

- The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
 - The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
 - If the above conditions are not met the test should be considered invalid and should be repeated.
- The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but 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.
 - Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. Calculations:

- Correction Factor:** The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
- Cutoff OD Value:** To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
(CF x Mean OD of Calibrator = Cutoff OD Value)
- Index Values/OD Ratios:** Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example: Mean OD of Calibrator	=	0.793
Correction Factor (CF)	=	0.25
Cutoff OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. Interpretations: Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

- An OD ratio ≤0.90 indicates no significant amount of IgM antibodies to *T. gondii* detected. A negative result indicates no current or previous infection with *T. gondii*. Such individuals are susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. When health care professionals suspect a primary infection, take another specimen within 7 days, and test concurrently in the same assay, with the original specimen, to look for seroconversion.
- An OD ratio ≥1.10 indicates that IgM antibodies specific to *T. gondii* were detected. A positive value indicates an active or recent infection with *T. gondii*.
The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antibody present.
- Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. If the repeat results are equivocal, collect and test another specimen within seven days with the original specimen. If the second specimen is equivocal (or negative), both specimens should be tested for the presence of *T. gondii*-specific IgG antibody. If the first specimen, or both specimens are positive for *T. gondii* IgG, it is likely that infection has occurred at an earlier date and residual IgM is being detected in the presence of IgG. If both samples are negative for *T. gondii* IgG, an early infection may be likely. Depending upon the acuteness of the clinical situation, another sample should be collected and assayed simultaneously with the first test samples, or an alternative methodology should be used.

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.
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. Results of the ZEUS ELISA *T. gondii* IgM Test System are not by themselves diagnostic; interpret only in light of the patients' clinical condition and the results of other diagnostic procedures.
2. Low levels of *T. gondii*-specific IgM antibodies may be detectable for up to one year following primary infection in some patients (1). Measurements of *T. gondii*-specific IgG antibodies may be of some value in the serological assessment of these patients.
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 (21, 22). Scientists do not understand the stimulus, or the significance of naturally occurring IgM antibodies directed against *T. gondii*.
6. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results on the ELISA Toxoplasma IgM.
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. Studies have reported false positive anti-Toxoplasma results for patients having autoimmune disease (23).
9. ZEUS Scientific did not validate the performance of the ZEUS ELISA *T. gondii* IgM Test System using neonatal samples.
10. A negative result for Toxoplasma IgM does not preclude the possibility of an acute infection in immunocompromised patients. *T. gondii*-specific IgG antibodies are generally low and *T. gondii*-specific IgM antibodies may be undetectable in patients who are immunocompromised (24).
11. 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 user's laboratory.
12. 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 (26 - 29).

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 four to six months (4). In some patients, IgM specific antibodies may be detectable for eight months to one year (1, 11). As part of the comparative study, an asymptomatic, "normal," population of 131 specimens was tested. A summary of this testing is shown in Table 3 and Table 4.

PERFORMANCE CHARACTERISTICS

1. Comparative Studies:

A comparative study was conducted to compare the ZEUS ELISA *T. gondii* IgM Test System to a commercially available IgM Capture ELISA for the detection of IgM antibodies to *Toxoplasma gondii*, in two different studies. The first study evaluated a total of 157 serum samples: 26 archived Toxoplasma IgM positive samples from a Toxoplasma reference laboratory, and 131 samples from normal plasma donors in the Southeastern United States. The presence of *T. gondii*-specific IgM in the samples from the reference laboratory was supported by the following information: Positive by Sabin-Feldman dye test, positive by reference laboratory, Toxoplasma IgM Capture ELISA, and clinical diagnosis. Evaluation of the samples occurred after treatment of the samples to remove IgG. Discrepant samples were evaluated using a commercial Toxoplasma IgM IFA test system. The results of the combined studies are summarized in Tables 1, 2, 3 and 4 below:

Table 1: ZEUS ELISA *T. gondii* IgM Test System

Relative Sensitivity	92.9%	(26/28)
Relative Specificity	100%	(122/122)
Relative Agreement	98.7%	(148/150)*

* Seven samples were equivocal by one or both ELISA test systems and excluded from the calculations.

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

		ZEUS ELISA <i>T. gondii</i> IgM Test System			
		Positive	Negative	Equivocal	Total
Commercial ELISA Toxoplasma IgM (Capture)	Positive	25	1	0	26
	Negative	0	0	0	0
	Equivocal	0	0	0	0
	Total	25	1	0	26

Relative Sensitivity = 96.2% (25/26)

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 3: Normal Plasma Donor Samples (n=131)

		ZEUS ELISA <i>T. gondii</i> IgM Test System			
		Positive	Negative	Equivocal	Total
Commercial ELISA Toxoplasma IgM (Capture)	Positive	1 (a)	10 (b)	0	11
	Negative	0	113	0	113
	Equivocal	0	7 (c)	0	7
	Total	1	130	0	131

(a) - Toxoplasma IgM positive by IFA

(b) - Discrepant results - see Table 4

(c) - Equivocal results excluded from calculations

Table 4: Analysis of Discrepant Normal Plasma Donor Results

Sample I.D.	ZEUS ELISA <i>T. gondii</i> IgM Test System	Commercial Toxoplasma IgM ELISA	Commercial IFA
22	-	+	+
49	-	+	-(p)*
65	-	+	-(p)
87	-	+	-(p)
123	-	+	-(p)
124	-	+	-(p)
128	-	+	-(p)
129	-	+	-(p)
130	-	+	-(p)
132	-	+	-(p)

* Polar staining reaction - not specific for *T. gondii* antibody

ZEUS analysis of this asymptomatic, normal population resulted in a relative specificity of 100% (113/113) using the ZEUS ELISA *T. gondii* IgM Test System.

Re-evaluation occurred upon the resolution of the 10 discrepant samples by IFA. Table 1 shows the relative sensitivity, specificity, and agreement of the ZEUS ELISA Toxoplasma IgM Test System.

A second study was performed to assess the performance of the ZEUS ELISA *T. gondii* IgM Test System using 19 unknown samples suspected of containing *T. gondii*-specific IgM antibodies. The results of this study are shown in Table 5.

Table 5: Evaluation of Unknown Samples

		ZEUS ELISA Toxoplasma IgM			
		Positive	Negative	Equivocal	Total
Commercial ELISA Toxoplasma IgM (Capture)	Positive	15	2*	0	17
	Negative	0	2	0	2
	Equivocal	0	0	0	0
	Total	15	4	0	19
*IFA Positive					
Relative Sensitivity = 82.2% (15/17)		Relative Specificity = 100.0% (2/2)		Percent Agreement = 89.5% (17/19)	

2. Precision and Reproducibility:

Technicians tested six specimens, with OD ratio values in the Positive, Calibrator, and Negative ranges, to determine intra-assay and inter-assay variation. On each of three days, a technician tested each specimen once a day, eight times each, resulting in 24 test points. A responsible party then calculated the mean OD ratio and coefficient of variation from the resulting data. Depiction of the results of the experiment is below in Table 6.

Table 6:	Intra-Assay (n=8)						Inter-Assay/ (n=3)	
	Run 1		Run 2		Run 3			
Serum	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
1 HP	4.89	3.5	4.17	6.2	5.51	3.2	4.86	4.3
2 HP	4.10	8.3	4.68	6.7	5.02	4.5	4.60	6.5
3 MP	2.07	8.7	2.42	2.5	2.72	9.6	2.40	6.9
4 LP	1.60	14.6	1.66	13.6	1.99	15.3	1.75	14.5
5 N	0.207	9.6	0.177	11.1	0.206	16.9	0.197	12.5
6 N	0.105	12.8	0.074	27.2	0.093	2.6	0.091	2.2

3. Cross Reactivity:

Cross reactivity studies were conducted to assess interference in the test procedure by rheumatoid factor (RF), EBV-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 ELISA Toxoplasma IgM Assay. One sample, strongly positive for Toxoplasma IgG (ELISA Ratio = 8.006) and rheumatoid factor (1:160) produced a low to mid positive IgM result (ELISA Ratio = 1:822) when tested in the ZEUS ELISA *T. gondii* IgM Test System prior to IgG absorption. Following IgG absorption, the ZEUS ELISA *T. gondii* IgM Test System result was clearly negative (ELISA Ratio = 0.160). Forty-six of 48 ANA samples were negative when tested with the ZEUS ELISA *T. gondii* IgM Test System. One of the positive samples exhibited a fine speckled pattern and a titer >1:1280. The other sample exhibited a nuclear pattern (1:160) and anti-Golgi activity (1:80). These studies indicate that interference with the test procedure by RF, EBV-IgM and ANA are minimal.

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ZEUS Scientific

200 Evans Way, Branchburg, New Jersey, 08876, USA

Toll Free (U.S.): 1-800-286-2111, Option 2

International: +1 908-526-3744

Fax: +1 908-526-2058

Website: www.zeusscientific.com

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For US Technical Support contact ZEUS Scientific, call toll free
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