ImmunoFA™

TOXO IgM TEST

For *In Vitro* Diagnostic Use

INTENDED USE

The ImmunoFA Toxo IgM Test is an immunofluorescence test for the presumptive detection and titration of IgM antibodies to *Toxoplasma gondii* in human serum and is presumptive for the diagnosis of acute, recent, or reactivated *T. gondii* infection. It is intended that the ImmunoFA Toxo IgG Test be performed in conjunction with this assay. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

BACKGROUND INFORMATION

Toxoplasma gondii is considered to be a significant pathogen of man and animals [1]. *T. gondii* is an obligate intracellular protozoan parasite, a coccidian, with world-wide distribution [2]. In the United States, serological studies have shown the incidence of antibody to *T. gondii* to range from 3% to 40% depending on the age group and geographic area surveyed [3, 4].

Toxoplasmosis in the immunocompetent individual is usually asymptomatic. Acute infection induces both humoral and cell-mediated immune responses which control the infection. With the appearance of the immune response, certain of the toxoplasma organisms become encysted. There is little or no evidence of host response to the cysts and they may remain dormant for many years.

When toxoplasma infection is acquired during pregnancy, there is a significant risk of infection to the fetus [1, 5]. It is generally agreed that congenital infection takes place only when acute infection is acquired during pregnancy. Women who have serological evidence of toxoplasma infection prior to becoming pregnant rarely, if ever, have infected neonates; it is the seronegative woman who becomes infected and seroconverts during pregnancy who may give birth to an infected infant [6].

Toxoplasmosis has emerged as a serious complication in the immunocompromised host, particularly in patients undergoing immunosuppressive therapy [7]. These individuals exhibit more severe infections upon primary exposure than do normal individuals and, if chronically infected, are more likely to undergo endogenous reinfection.

Primary infection with *T. gondii* is accompanied by the production of antibody reactive with the organism. Antibodies of the IgM class appear within the first week following infection, peak in 3 to 4 weeks, and generally become undetectable within 3 to 4 months. Exceptions to this general pattern of IgM production have been noted in the form of early (3 week) loss of detectable IgM or persistence of low titers of IgM for one year or more [8]. IgG antibody to toxoplasma usually becomes detectable within 3 weeks following primary infection and peaks between 2 to 6 months, depending on the serological test used for detection [1]. Once peak titers occur, they decrease slowly and persist at detectable levels throughout life.

The indirect immunofluorescence method possesses certain advantages over other methods in the measurement of specific IgM antibody. By utilizing a fluorescein-conjugated antiglobulin specific for human IgM, it may be possible to detect antibody without separation of the IgG and IgM fractions of the test serum. The test is easy to perform and the results can be obtained in a few hours.

RATIONALE OF THE TOXO IgM TEST

The Toxo IgM Test utilizes the indirect fluorescent antibody technique for the detection and titration of IgM antibody to toxoplasma in human serum. The antigen substrate consists of *T. gondii* strain RH, dried on microscope slides. The organisms are fixed and no infective forms can be detected using *in vivo* inoculation methods. Test serum is applied to the antigen substrate and incubated at 37 °C. Following incubation, the serum is rinsed from the slide and fluorescein-conjugated antihuman IgM is applied. Following the second incubation the slide is rinsed and examined under a fluorescence microscope. If IgM antibody to *T. gondii* is present in the test serum, it will combine with the antigens of the fixed organisms and the fluorescein-conjugated antiglobulin will be bound causing the organisms to fluoresce. The reaction is considered positive when a majority of the fixed toxoplasma organisms exhibit fluorescence around their entire periphery.

When combined with the ImmunoFA Toxo IgG Test Kit, the Toxo IgM Test is a simple, rapid method that is useful in determining the presence of acute toxoplasma infection early in its course. Such rapid detection of acute infection in at-risk populations such as pregnant women, immunocompromised patients, or newborn infants allows for the rapid institution of appropriate therapy which can significantly reduce the morbidity and mortality associated with toxoplasma infection in these patients.

MATERIALS REQUIRED BUT NOT PROVIDED

37 °C incubator

Humidified chamber or petri dish

Distilled water

Glassware for diluting PBS

Squeeze bottle

Absorbent tissue

Cotton tip applicators (swabs)

Serological pipettes, Pasteur pipettes, and rubber bulbs

Small test tubes for serum dilutions

Mounting Fluid (Product No. 1025)

Coverslips (22 x 50 mm)

Fluroescence microscope

REAGENT PREPARATION AND STORGE

Each Toxo IgM Test contains ten 10-well *Toxoplasma gondii* antigen slides, IgM positive and IgM negative control sera, FITC-conjugated antihuman IgM with Evans Blue counterstain, and phosphate buffered saline solution. When stored at refrigerator temperatures, the kit, in its original packaging, is good until the date indicated on the package label.

Some assay components contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

10X PBS. A 10X concentrate of 0.01M phosphate buffered saline (pH 7.2-7.6) with 0.1% NaN_3. Store at room temperature to avoid crystallization. If crystallization occurs during storage in the cold, gently heat to 37 °C to dissolve the crystals. Dilute 1:10 with distilled water for use. Store 1X PBS refrigerated.

***Toxoplasma gondii* Antigen Slides.** Ten 10-well microscope slides with dried, formalin-fixed *T. gondii*, strain RH, harvested from mouse peritoneal fluid are provided. The slides are contained in a plastic insert and packaged in buffered glycerol (30%) preservative. *Toxoplasma gondii* antigen slides (Product 1201) should be refrigerated. DO NOT FREEZE. Slides should be kept wet at all times by replacing preservative, if necessary, with buffered glycerol. Buffered glycerol for replacing preservative is prepared by mixing 3 parts glycerol with 7 parts 1X PBS. Frozen slides (Products 1206 and 1207) are not shipped in buffered glycerol. They should be stored below -20 °C.

IgM Positive Control. Human sera containing IgM antibody to *T. gondii* at 1:8 working dilution.

IgM Negative Control. Human serum having no IgM antibody but containing IgG antibody to *T. gondii* at a 1:8 working dilution.

> **NOTE:** Each donor unit used in the preparation of positive and negative controls was tested by an FDA approved method for the presence of the antibody to HIV as well as to hepatitis B surface antigen and found to be negative (were not repeatedly reactive).

IgM Conjugate. Contains affinity purified goat antihuman IgM (mu) conjugated with fluorescein isothiocyanate (FITC) in buffered diluent containing carrier protein, Evans Blue, and 0.1% NaN_3. This product is prepared ready for use with the Toxo IgM Test and the fluorescence microscope system described in this insert. Each laboratory should confirm the most suitable working dilution in its system using the positive

control serum. Evans Blue has been added to the conjugate as a counterstain to mask autofluorescence of the toxoplasma organisms. A low concentration of the dye has been used to minimize the masking of specific fluorescence. The conjugate should be stored refrigerated, and should not be subjected to repeated freezing and thawing.

PREPARATION OF PATIENT SERA

Patient sera should be clear and free from obvious contamination. For best results, lipemic or hemolyzed sera should not be used. Although some meaningful information may be gathered from a single serum sample, especially in at-risk populations, paired serum samples (acute and convalescent) should also be collected, stored, and run simultaneously for more meaningful information. Serum may be refrigerated up to 5 days before testing but is best stored below -20 °C.

Warning - Potential Biohazardous Material. Because no test method can offer complete assurance that Human Immunodeficiency Virus (HIV), hepatitis B virus, or other infectious agents are absent, this specimen/reagent should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen [9].

TEST PROCEDURE

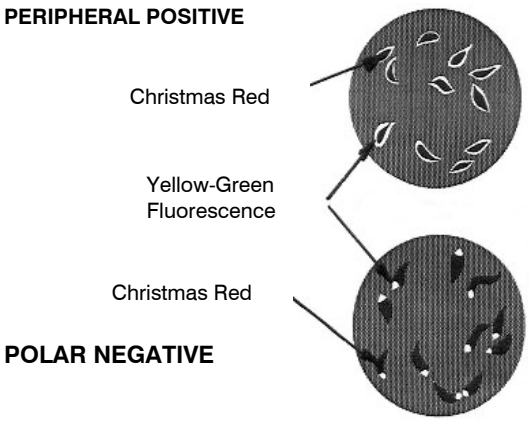
1. The choice of dilution scheme and controls run with each test rests with the individual laboratory. At GenBio, the following procedures and dilution schemes are used. The 1:8 working dilution of negative control is run with each batch of tests. The 1:8 working dilution of positive control is titered to one dilution above the titer indicated on the vial label. All dilutions are prepared with 1X PBS. Sera are tested at two-fold serial dilutions from 1:8 (1:2 for a neonate) to 1:256 or until an endpoint is reached. It is recommended that the decision to perform a test for Toxo IgM antibody be based on the presence of Toxo IgG antibody. If the Toxo IgG test is negative, performance of a Toxo IgM test is not recommended due to difficulty of interpretation when no IgG antibody is present to confirm infection.
2. Remove the desired number of slides from the vial by pulling up on the plastic tab and lifting the insert partially out of the vial to expose the frosted end of the slides. Rinse each slide 10 to 15 seconds under slowly running cool tap water then, holding the slide upright and using a squirt bottle, gently wash areas around wells with 1X PBS. Do not squirt buffer directly at the wells, but do allow PBS to run over the wells. It is essential that the buffered glycerol preservative be thoroughly removed. Frozen slides need not be rinsed, but can be made ready for use by thawing in 1X PBS for 5 min. Keep slides wet until ready for use by immersing them in a petri dish or Coplin jar containing 1X PBS.
3. Wipe the back of the slide dry and gently blot the surface with absorbent tissue. DO NOT RUB. The blotting may be best accomplished by inverting the slide onto absorbent tissue and gently tapping the back of the slide once or twice. The area between the wells must be dry to prevent cross-mixing. This may be accomplished by wiping between the wells with a cotton tip applicator.
4. Place the slide in a humidified incubation chamber. (Moistened absorbent paper in a petri dish works well as a humidified chamber. For a large number of slides a plastic box or glass baking dish with a cover serves well as a humidified chamber).
5. Add 1 drop (approximately 30 μL) of diluted serum to each well. The volume should be sufficient to prevent dehydration during incubation but the wells should not be allowed to overflow. If cross-mixing of the wells occurs at this time, quickly wash the slide with 1X PBS and start the test again. This will not affect the reactions.
6. Cover the chamber and incubate at 37 °C for 30 minutes. (A sixty-minute incubation may be used especially in those sera having high concentrations of Toxo IgG antibody).
7. Remove chamber from incubator and rinse the slide gently but generously with 1X PBS 4 to 5 times without aiming the stream of PBS directly at the wells. Cross mixing during rinsing does not affect the test.
8. Dry the underside of the slide and gently blot the slide surface. DO NOT RUB.
9. Return the slide to the humidified chamber and cover each well with 1 drop (approximately 30 μL) antihuman IgM.
10. Return the chamber to the incubator for 30 minutes.
11. Remove chamber from incubator and rinse the slide gently but generously with 1X PBS 4 to 5 times without aiming the stream of PBS directly at the wells.
12. Gently blot the surface and wipe the back of the slide. Place a small drop of buffered glycerol mounting fluid on each well and place a 22 x 50 mm coverslip over the wells. Do not allow slides to dry before applying mounting fluid.
13. Examine the reactions under a fluorescence microscope using high power magnification (400X).

READING THE TOXO TEST

The Center for Disease Control (CDC) recommends the following:

4+	brilliant yellow-green fluorescence around the ENTIRE periphery of the organism; the internal red counterstain may be completely masked by radiating fluorescence.
3+	brilliant yellow-green fluorescence around the ENTIRE periphery of the organism; the internal red counterstain is visible.
2+	thin band of yellow-green fluorescence around the ENTIRE periphery of the organism; the internal red counterstain is very apparent.
1+	thin band of dull yellow-green fluorescence around the ENTIRE periphery of the organism; the internal red counterstain is very apparent.
±	barely visible incomplete band of dull, yellow-green fluorescence around portions of the periphery of the organism; the internal red counterstain is very apparent.

The endpoint is considered to be the highest dilution of test serum to show a thin band of dull yellow-green fluorescence (1+ fluorescence intensity) around the entire periphery of a majority of the organisms. The IgM negative control serum may exhibit polar staining which is caused by the nonspecific binding of IgM-class immunoglobulin to receptors on the polar end of the toxo organisms. Polar staining will be particularly intense in IgM testing because the conjugate is IgM specific. In a negative reaction, the fluorescence never extends around the entire cell periphery and the internal red counterstain is very apparent. The endpoint of the IgM positive control serum should be within one two-fold serial dilution of the titer given on the label. For example, if the tier of the IgM positive control is given as 1:64, the titer obtained may vary between 1:32 and 1:128.



INTERPRETATION OF RESULTS

Interpreting the significance of tests for Toxo IgM antibody requires consideration of several factors and additional testing may be necessary for an accurate interpretation. **It is recommended that both the IgG-IFA and IgM-IFA toxoplasma tests be performed. A positive IgM result should not be interpreted positive unless the IgG result is also positive.** The two most uncomplicated situations are: 1) the IgG-IFA titer is ≤1:128, the IgM-IFA test is negative and a diagnosis of chronic, long-standing Toxo infection is likely; and 2) the IgG-IFA titer is ≥1:512, the IgM-IFA titer is ≥1:64 and diagnosis or recently acquired Toxoplasma infection is almost certain. In patients with extremely elevated levels of Toxo IgM antibody a prozone reaction may be observed in the form of negative reactions at low (usually ≤1:32) dilutions with reactions becoming positive at higher (≥1:64) dilutions.

When the IgG-IFA titer is ≥1:512 and the IgM-IFA is positive with a titer of ≤1:64, infection is likely although confirmatory testing is recommended to rule out possible false positive IgM results that may occur in sera containing both rheumatoid factor and specific IgG antibody [10,11]. In the immunofluorescence staining procedure, IgM rheumatoid factor will combine with specific Toxo IgG antibody bound to the antigen and will react with the anti-IgM conjugate resulting in a false positive test for specific Toxo IgM antibody. This type of false positive reaction is also seen in sera from some newborns, both normal and congenitally infected, and is due to production of IgM antibody against passively acquired maternal IgG [12].

When the IgG-IFA titer is ≥1:512 and the IgM-IFA is negative, the possibility of a false negative IgM-IFA must be considered. Such false negative reactions are due to the blocking effect of high levels of specific IgG antibody on the binding of IgM to the antigen substrate so that the amount of specific IgM bound is not sufficient to yield a positive IgM-IFA test.

The ease with which difficult samples may be interpreted can be increased by confirmatory procedures such as fractionation of serum IgG and IgM or selective removal of IgG antibody [13]. Testing of the separated fraction can be performed without rheumatoid factor interference or competitive binding problems. Demonstration of a 4-fold or greater rise in IgG-IFA and/or IgM-IFA titer in a second serum sample drawn three weeks after the first and tested simultaneously with the first can confirm the presence of acute infection. Also, the occurrence of more than one positive specific IgM assay (i.e., positive IgM-IFA test for both Toxo and Herpes Simplex virus) in a single serum sample is an indication that results are falsely positive due to the presence of rheumatoid factor since positive IgM staining is obtained with all antigens to which IgG antibodies are present.

False positive Toxo IgM-IFA reactions may also occur in sera from patients having anti-nuclear antibody [14]. Serological tests serve as an aid to diagnosis and must be considered in relation to clinical findings.

The IgM-IFA test may rapidly provide information necessary to evaluate the possibility of congenital toxoplasma infection in the newborn infant. Parallel tests of serum samples from both the mother and the newborn are run using both the Toxo IgG Test (Product No.1200) and the Toxo IgM Test (Product No.1300). Interpretation of representative results from such tests are given below. *

Serum Source		Antihuman IgG Globulin	Antihuman IgM Globulin	Interpretation of Results of Newborn
Infant	S_1	16	Neg	Probably only passive transfer, the second specimen confirms, infection unlikely
	S_2	Neg	Neg	
Mother	S_1	16	Neg	
	S_2	16	Neg	
Infant	S_1	256	Neg	Probably only passive transfer, the second specimen confirms, infection ruled out
	S_2	16	Neg	
Mother	S_1	256	Neg	
	S_2	256	Neg	
Infant	S_1	64	4	Presence of IgM antibody in first serum indicates infection, rising anti-IgG titer confirms infection
	S_2	1024	Neg	
Mother	S_1	2048	Neg	
	S_2	1024	Neg	
Infant	S_1	128	16	IgM antibody in first serum drawn during first week of life indicates infection; falling titer in infant, rising titer in mother shows mother is infected, infant is not
	S_2	4	2	
Mother	S_1	1024	64	
	S_2	4096	64	
Infant	S_1	1024	16	IgM antibody in first serum drawn during first week of life indicates infection, rising IgM titer in infant shows infant is infected
	S_2	256	64	
Mother	S_1	1024	4	
	S_2	1024	4	

*Adapted from Palmer, DF, JJ Cavallaro, K Herrmann, JA Stewart, and KW Walls. US Dept. HEW Immunology Series No.5, Procedural Guide (1974). S_1 sera drawn at time of delivery, S_2 sera drawn 3 weeks later.

SUGGESTED FLUORESCENCE MICROSCOPE SYSTEM

The following microscope system is used to standardize these fluorescence reagents: (It may be necessary for you to restandardize these reagents for use in your microscope system.) A Zeiss fluorescent microscope equipped with a 10X eyepiece, 16X and 40X objectives, Epi-illuminator with 100W halogen lamp, FITC excitation filter (KP490) and yellow absorbing filter (LP530).

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