ImmunoFA™

TOXO IgG TEST

For *In Vitro* Diagnostic Use

INTENDED USE

The ImmunoFA Toxo IgG Test is an immunofluorescence test for the detection and titration of IgG antibodies to Toxoplasma gondii in human serum. 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 worldwide 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.

Serological tests are the primary method used to diagnose toxoplasma infection. Several serological assays may be used to demonstrate toxoplasma antibody, including the Sabin-Feldman dye test [9], the complement fixation test [10], the indirect hemagglutination test [11], and the indirect immunofluorescent antibody (IFA) test [12]. Excellent correlation exists between relative titers obtained in the IFA test and those obtained using the longer, more laborious procedures. In addition, the IFA test detects both IgG and IgM-class antibody. If the test is positive using antihuman IgG, a monospecific antihuman IgM fluorescein conjugate may be employed to distinguish the early antibody response characteristic of primary infection.

RATIONALE OF THE TOXO TEST

The Toxo IgG Test utilizes the indirect fluorescent antibody technique for the detection and titration of toxoplasma antibody in human sera. 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 IgG is applied. Following a second incubation, the slide is rinsed and examined under a fluorescence microscope. If antibody to *T. gondii* is present in the test serum, it will combine with the antigens of the fixed organisms and the fluorescein-conjugated anti-IgG will be bound causing the organisms to fluoresce. The reaction is considered positive when the majority of the fixed toxoplasma organisms exhibit fluorescence around their entire periphery.

The IgG Test employing fluorescein-conjugated antihuman IgG is a simple, rapid method for the screening of patient sera to detect those individuals who have had previous infection (positive reactors), those who are susceptible to infection (negative reactors), and those with acute infections (demonstration of a rising titer to high levels in paired serum samples).

When combined with the ImmunoFA Toxo IgM Test, the Toxo IgG Test 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 STORAGE

Each Toxo IgG Test contains ten 10-well *Toxoplasma gondii* antigen slides, high positive, low positive, and negative control sera, FITC-conjugated antihuman IgG with Evans Blue counterstain, and phosphate buffered saline solution. When stored at refrigerator temperatures, the kit is stable 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.

Positive Controls. Two human sera containing IgG antibody to *T. gondii* are provided: a high titer positive and a low titer positive as labeled. Both sera are provided at 1:16 working dilution.

Negative Control. Human serum having no antibody to *T. gondii* is provided at a 1:16 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).

IgG Conjugate. Contains affinity purified goat antihuman IgG (gamma) 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 IgG Test and the fluorescence microscope system described in this insert. Each laboratory should confirm the most suitable working dilution in its system using high and low positive control sera. 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 population, 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 the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.

TEST PROCEDURE

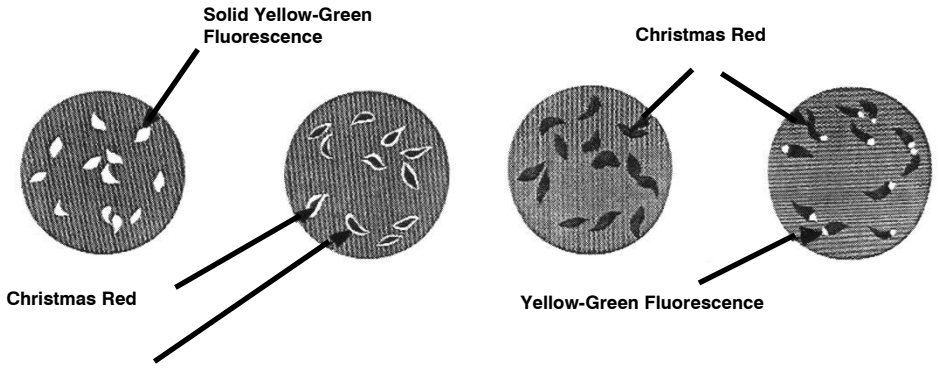
1. The choice of dilution scheme and controls run with each test rests with the individual laboratory. At GenBio, the following procedures and dilution schemes are used. The 1:16 working dilution of negative control is run with each batch of tests. The 1:16 working dilutions of high and low titer positive controls are titered to one dilution above the titer indicated on the vial label. Test sera are screened at 1:16 and 1:256 dilutions. Sera that are positive at 1:256 dilution are further tested at two-fold serial dilutions until an endpoint is reached (usually 1:8192). All dilutions are prepared in 1X PBS.
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.
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 IgG.
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 negative control serum may either exhibit no yellow-green fluorescence or polar staining may occur. 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 positive control sera should be within a two-fold serial dilution of the titer given on the label. For example, if the titer of the low positive control is given as 1:64, the titer obtained may vary between 1:32 and 1:128.



In most instances, no yellow-green fluorescence is seen. When fluorescence is seen, it is usually restricted to one end of the organism (POLAR STAINING). It never extends all around the cell periphery. The red counterstain is very apparent.

INTERPRETATION OF RESULTS

Results of Screen		
1:16	**1:256**	**Interpretation**
Negative	Negative	No toxoplasma antibody present. Indicates that patient is at risk of acquiring acute toxoplasma infection. Consideration should be given to monitoring for seroconversion in high-risk patients such as the pregnant female or the immunocompromised patient who do not have toxoplasma antibody. Such monitoring can reduce the potentially severe consequences of acute infection in these patients by allowing rapid institution of specific therapy.
Positive	Negative	This finding usually reflects past exposure to toxoplasma but may also indicate the early stages of acute infection. In at-risk populations (e.g., the pregnant female, the neonate, or the immunocompromised patient) or if clinical evidence of acute infection is present, an IgM-IFA should be performed. The presence of a positive IgM-IFA is suggestive of acute infection although in a small percentage of individuals the test may remain positive for years. The presence of acute infection may be confirmed by demonstrating 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 sample.
Positive	Positive	This finding reflects either past exposure to toxoplasma or acute infection. The serum should be further titrated to establish an endpoint. In at-risk populations or if clinical evidence of acute infection is present, an IgM-IFA test should be run and a second serum should be drawn 3 weeks after the first sample for parallel testing. If the IgG-IFA titer is ≥ 1:512 and the IgM-IFA titer is ≥1:64, diagnosis of recently acquired toxoplasma infection is almost certain. If the IgG-IFA titer is ≥ 1:512 and the IgM-IFA titer is < 1:64, diagnosis of recent infection is likely. If the IgM-IFA test is negative but a four-fold or greater rise in IgG-IFA titer occurs, the patient has had a recent infection. If the IgM-IFA test is negative and a four-fold or greater rise in IgG-IFA titer does not occur, the patient has chronic toxoplasma infection.

NOTE: False positive reactions may occur in sera containing high levels of rheumatoid factor [13] or in sera from patients having antinuclear antibody [14]. Serological tests serve as an aid to diagnosis and must be considered in relation to clinical findings.

The IgG-IFA and IgM-IFA tests 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 or representative results from such tests are given below.* Recommended serum dilutions for the newborn begin at 1:2.

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

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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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