T. gondii IgG Test System

IVD

REF FA8001G

Rx Only

INTENDED USE

The ZEUS IFA *Toxoplasma gondii* IgG Test System is designed to detect the presence of circulating *T. gondii* antibodies in human sera and is for *In Vitro* diagnostic use. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

SIGNIFICANCE AND BACKGROUND

Toxoplasmosis is an infectious disease caused by an obligate intracellular parasite *T. gondii*. Toxoplasmosis may be associated with many symptoms that are frequently nonspecific, such as adenopathy, splenomegaly, jaundice, fever, vomiting, etc. Because of the wide range of clinical manifestations, toxoplasmosis should be considered in the differential diagnosis of most patients who present with the above symptoms, especially during early infancy (1 - 2).

Toxoplasmosis is a common latent infectious agent (3). Since immunosuppressed transplant patients frequently develop latent infections, toxoplasmosis should be considered in the differential diagnosis of any immunosuppressed patient who develops clinical or laboratory evidence of central nervous system damage (3). Several test procedures have been employed to detect *T. gondii* antibodies in patient sera. The indirect fluorescent antibody (IFA) procedure is one of the most widely accepted Toxoplasma test methodologies because of its ability to detect antibodies to *T. gondii* cell-wall constituents (4).

In addition, the IFA procedure can easily be adapted to detect early IgM antibodies (5 - 6) which are useful in assessing the acute phase of toxoplasmosis. The IFA procedure has been shown to have excellent reproducibility and correlation with the standard Sabin-Feldman dye test (7). Other methodologies for detecting toxoplasma antibodies include the complement fixation, indirect hemagglutination, and more recently, ELISA techniques.

Toxoplasmosis was first isolated from birds, reptiles, and mammals in 1908 (8 - 9). Sources of toxoplasma infections include:

1. Human
   a. Mother to fetus.
   b. Immunosuppression following organ transplantation.
   c. Blood transfusions.
2. Animal
   a. Through ingestion of improperly cooked meat.
   b. From soil contaminated with oocysts from domestic or feral cat feces.
3. Through factors such as socioeconomic environment, age, sex, or other undefined factors.

PRINCIPLE OF THE ASSAY

The ZEUS IFA *T. gondii* IgG Test System is a standardized assay designed to detect the presence of circulating *T. gondii* antibodies in human sera. The assay employs *T. gondii* substrate (antigen) affixed to multiwell substrate slides and goat anti-human immunoglobulin adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. Step one is the interaction of *T. gondii* antibodies in patient sera with the *T. gondii* organisms.
2. Step two is the interaction of FITC labeled anti-human IgG with Toxoplasma antibodies attached to the *T. gondii* organisms producing apple-green staining in a positive assay (see Assay Procedure).

The ZEUS IFA *T. gondii* IgG Test System is specific, relatively rapid, sensitive, and provides a useful alternative method to assay Toxoplasma antibodies in patient sera.

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, each well contains formalin-fixed *T. gondii* organisms (RH strain). Each well contains 50-100 organisms per high power field (400X) for optimum reactivity and readability. Also includes a desiccant pouch.
CONJ	2.	Conjugate: Goat Anti-human IgG (γ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL + 1	3.	High Positive Control (Human Serum): Will produce 3+ to 4+ positive apple-green staining around the periphery of the organisms. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL + 2	4.	Low Positive Control (Human Serum): Will exhibit at least 2+ positive apple-green staining around the periphery of the organisms. One, 0.5mL, blue-capped, vial. Ready to use.
CONTROL -	5.	Negative Control (Human Serum): Characterized by the absence of staining around the periphery of the organisms. One, 0.5mL, green-capped, vial. Ready to use.
DIL SPE	6.	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	7.	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	8.	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. 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).

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 (11, 12). 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 (14).

STORAGE CONDITIONS

2°C – 8°C	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	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**. Prepare a 1:16 dilution (e.g.: 10µL of serum + 150µL of SAVe Diluent® or PBS) of each patient serum. **The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**
   Dilution Options:
   a. As an option, users may prepare initial sample dilutions using PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles).
   b. 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.
   c. When titrating patient specimens, initial dilutions should be prepared in SAVe Diluent®, PBS, or Zorba-NS and all subsequent dilutions should be prepared in SAVe Diluent® or PBS only. Titrations should not be prepared in Zorba-NS.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash slides for two, 5 minute intervals, changing PBS between washes.
7. Remove Slides from PBS. Rinse Slides briefly with deionized or distilled water and air dry Slides. Do not disturb the organisms in the wells.
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. 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, the Positive Controls, 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 yellow-green staining along the periphery of the *T. gondii* substrate, and/or when the organism has a red appearance. it is also considered a negative reaction if only a segment of the organism fluoresces yellow-green, and not the entire periphery. Polar staining or a beaded fluorescent pattern is a non-specific reaction and should be considered negative. The reactions of the negative control may be used as a guide for interpreting patient samples.
   b. High Positive Control - characterized by a 3+ to 4+ yellow-green fluorescent staining intensity along the entire periphery of the organism.
   c. Low Positive Control – characterized by at least a 2+ yellow-green fluorescent staining intensity in the same manner as the High Positive Control.
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

Examine the slides to ascertain the relative staining intensity of the *T. gondii* substrate organisms. A negative reaction is one that demonstrates no staining along the periphery of the substrate organisms or one that shows only polar staining of the substrate organisms. Positive reactions are observed as confluent linear staining 1+ to 4+ along the periphery of the substrate organisms. A 1+ reaction is one that shows weak but distinct apple-green peripheral staining and represents the endpoint reaction in a titration. A 4+ reaction is one that shows very strong apple-green staining at the periphery of the substrate organisms. Do not prepare serial dilutions for endpoint titers in **Zorba-NS.**

Titer	Result
Negative	Non-Diagnostic
1:16 - 1:64	Usually reflects only some past exposure, or may represent early disease with a rising titer.
1:256	Usually indicates relatively recent exposure or current involvement. Clinician should be alerted.
1:1024 or higher	Very significant. The clinician should be advised to consider toxoplasmosis and to further attempt to identify the disease.

LIMITATIONS OF THE ASSAY

1. False positive reactions may occur with sera from patients with high titer rheumatoid factor (6) and antinuclear antibodies (10).
2. A single test by itself is not diagnostic. The results of this assay should be interpreted in the light of the patients history, physical exam, and clinical symptoms by a medical authority.

EXPECTED RESULTS

The expected value in the normal population is negative. However, apparently healthy individuals sera may frequently contain toxoplasma antibodies at low titer and occasionally at high titers.

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