Introduction and Background

A. Overview

Toxoplasma gondii causes toxoplasmosis, a common disease that affects 30-50 of every 100 people in North America by the time they are adults. The mean source of infection is direct contact with cat feces or from eating undercooked meats. Toxoplasmosis generally presents with mild symptoms in immunocompetent individuals; in the immunocompromised patient, however, the infection can have serious consequences. Acute toxoplasmosis in pregnant women can result in result in miscarriage, poor growth, early delivery or stillbirth. Treatment of an infected pregnant woman may prevent or lessen the disease in her unborn child. Treatment of an infected infant will also lessen the severity of the disease as the child grows. IgG and IgM antibodies to Toxoplasma can be detected with 2-3 weeks after exposure. IgG remains positive, but the antibody level drops overtime. ELISA can detect Toxoplasma IgM antibody after one year after infection in over 50% of patients. Therefore, IgM positive results should be evaluated further with one or two follow up samples if primary infection is suspected.

B. Test Principle

Diluted patient serum is added to wells coated with purified Toxoplasma antigen. Toxoplasma IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample.

C. Notice for Application of Kit

This kit has been configured for research use only and is not for diagnostic and clinical use.

D. Application

The Toxoplasma IgA ELISA Kit is intended for the detection of IgA antibody to Toxoplasma in human serum or plasma.

Material and Method

A. List of component

	96 Tests
Microwells coated with Salmonella typhi antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1.5 ml
Positive Control: 1 vial (ready to use)	1.5 ml
Negative Control: 1 vial (ready to use)	1.5 ml
Enzyme conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
Wash concentrate 20X: 1 bottle	25 ml

B. Additional Required Materials But Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. Micortiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

C. Stability and Storage

1. Store the kit at 2-8℃.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

D. Warnings and Precautions for Users

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is designed for research use only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

E. Specimen Collection and Handling

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8℃ for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

F. Reagent Preparation and Storage

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26℃).

G. Protocol

Bring all specimens and kit reagents to room temperature (18-26℃) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the mean values of each sample by cut-off value.

- Example of typical results:

  Calibrator mean OD = 0.8

  Calibrator Factor (CF) = 0.5

  Cut-off Value = 0.8 x 0.5= 0.400

  Positive control O.D. = 1.2

  Ab Index = 1.2 / 0.4 = 3

  Patient sample O.D. = 1.6

  Ab Index = 1.6 / 0.4 = 4.0

Quality Control

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

Interpreatation

The following is intended as a guide to interpretation of Toxoplasma IgA test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- Antibody Index Interpretation

<0.9	No detectable IgA antibody to Toxoplasma.
0.9-1.1	Borderline positive. Follow-up testing is recommend if clinically indicated
>1.1	Detectable IgA antibody to Toxoplasma.

Limitations of the Test

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

References

1. Wilson M; Remington JS; Clavet C; Varney G; Press C; Ware D. Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to Toxoplasma gondii. The FDA Toxoplasmosis Ad Hoc Working Group.

2. Obwaller A; Hassl A; Picher O; Asp¨ock H. An enzyme-linked immunosorbent assay with whole trophozoites of Toxoplasma gondii from serum-free tissue culture for detection of specific antibodies. Parasitol Res 1995;81(5):361-4.
3. Loyola AM; Durighetto AF Jr; Silva DA; Mineo JR. Anti-Toxoplasma gondii immunoglobulins A and G in human saliva and serum. J Oral Pathol Med 1997; 26(4):187-91.
4. Doehring E; Reiter-Owona I; Bauer O; Kaisi M; Hlobil H; Quade G; Hamudu NA; Seitz HM. Toxoplasma gondii antibodies in pregnant women and their newborns in Dar es Salaam, Tanzania. Am J Trop Med Hyg 1995;52(6):546-8.
5. Cotty F; Descamps P; Body G; Richard-Lenoble D. Prenatal diagnosis of congenital toxoplasmosis: the role of Toxoplasma IgA antibodies in amniotic fluid [letter]. J Infect Dis 1995;171(5):1384-5.
6. Altintas N; Kuman HA; Akisu C; Aksoy U; Atambay M. Toxoplasmosis in last four years in Agean region, Turkey. J Egypt Soc Parasitol 1997;27(2):439-43.