TOXOPLASMA IgG ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1085

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ENZYME IMMUNOASSAY FOR THE DETECTION OF IgG ANTIBODIES TO TOXOPLASMA GONDII IN HUMAN SERUM

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Toxoplasma IgG Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40

5 µl / 200 µl

2. Three incubations at 37°C

Diluted Sample 100 µl	Enzyme Conjugate 100 µl	TMB Reagent (One-Step) 100 µl
30 min.	30 min.	15 min.

3. Stop with 100 µl of acid. Read O.D. at 450 nm

INTENDED USE

The Toxoplasma IgG ELISA is intended for use in evaluating a patient's serologic status to *Toxoplasma gondii* infection.

INTRODUCTION

Toxoplasmosis is caused by the intracellular parasite *Toxoplasma gondii* and may be contracted by consuming contaminated meat or by coming in contact with cat feces containing oocysts. In adolescence and adulthood, most infections are subclinical. However, if a pregnant woman contracts toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation. Asymptomatic infants may develop abnormalities later in life. The Toxoplasma IgG ELISA is an accurate serologic method to detect Toxoplasma IgG antibody for clinical identification of toxoplasmosis.

PRINCIPLE OF THE TEST

Purified *Toxoplasma gondii* antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the *Toxoplasma gondii* IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Microtiter Wells: Toxoplasma antigen-coated wells (12x8 wells)
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml)
- Sample Diluent (green color): 1 bottle (22 ml)
- Negative Calibrator: 0 IU/ml. Natural cap. (150 µL/vial)
- Cut-off Calibrator: 32 IU/ml. Yellow cap. (150 µL/vial)
- Positive Calibrator: 100 IU/ml. Red cap. (150 µL vial)
- Positive Calibrator: 300 IU/ml. Green cap. (150 µL vial)
- Negative Control: Range stated on label. Blue cap. (150 µL vial)
- Positive Control: Range stated on label. Purple cap. (150 µL vial)
- Wash Buffer Concentrate (20x): 1 bottle (50 ml)
- TMB Reagent (One-Step): 1 vial (11 ml)
- Stop Solution: 1N HCl. Natural cap. 1 vial (11 ml)

STORAGE OF TEST KITS AND INSTRUMENTATION

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

WARNING AND PRECAUTIONS

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, as 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components from different lots should not be mixed.
4. This product contains components 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.

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrators by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrators, 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.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*

Note: Make sure there are no air bubbles in each well before reading.

13. Read O.D. at 450 nm ***within 15 minutes*** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate cut-off (32 IU/ml) calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and patient samples (X_s).
3. Calculate the Toxoplasma IgG Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c.

Example of typical results: Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

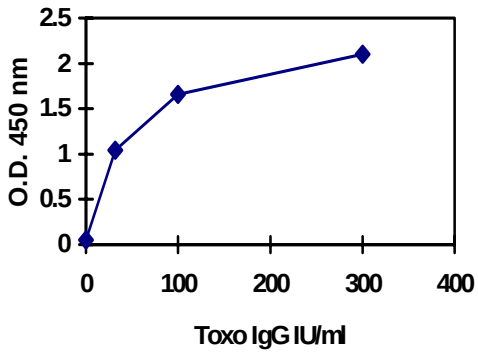
Cut-off Calibrator Toxoplasma G Index = 1.0

1. Cut-off Calibrator (32 IU/ml) O.D. = 0.901, 0.909 $x_c = 0.905$
2. Negative Control O.D. = 0.097, 0.094 $x_n = 0.096$
   Toxo G Index = 0.096 / 0.905 = 0.11
3. Positive Control O.D. = 1.882, 1.921 $x_p = 1.901$
   Toxo G Index = 1.901 / 0.905 = 2.10
4. Patient Sample O.D. = 2.459, 2.392 $x_s = 2.426$
   Toxo G Index = 2.426 / 0.905 = 2.68

QUANTITATIVE ESTIMATION OF TOXOPLASMA IgG

For a quantitative determination of anti-Toxoplasma IgG levels of positive specimens in IU/ml, OD of cut-off and positive calibrators are plotted on Y-axis in graph versus their corresponding anti-Toxoplasma IgG concentration of 0, 32, 100, and 300 IU/ml on X-axis. The Toxo IgG levels in patient sera are read off the graph using their individual OD values. For example:

Toxo IgG (IU/ml)	A_{450}
0	0.051
32	1.040
100	1.659
300	2.103



Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

QUALITY CONTROL

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Toxo IgG index for Negative and Positive Controls should be in the range stated on the Certificate of Analysis.

INTERPRETATION

Negative: Toxo G Index less than 0.90 indicates absence of prior exposure to Toxoplasma (< 32 IU/ml).

Equivocal: Toxo G Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: Toxo G Index of 1.00 or greater, or WHO IU/ml value greater than 32 IU/ml is seropositive. It indicates prior exposure to the Toxoplasma virus.

If current infection is suspected, a second sample obtained 8-14 days later should be tested for IgG antibody simultaneously. Toxo G Index ratio between paired samples greater than 1.5 is highly suggestive of a significant rise in antibody. It may be considered as indicative of acute Toxoplasma infection.

STANDARDIZATION AND ASSIGNMENT OF CUT-OFF VALUE

The Reference Standards are calibrated against the World Health Organization's Third International Standard for Anti-Toxoplasma in human serum. Code: TOXM. A cut-off calibrator of 32 IU/ml is used based on this standization.

PERFORMANCE CHARACTERISTICS

I. Specificity and Sensitivity:

A total of 188 patient samples were used to evaluate specificity and sensitivity of the test. The Toxo IgG ELISA test results were compared to a commercial ELISA kit results:

		Reference Toxo IgG ELISA			
		N	E	P	Total
Toxo IgG ELISA	N	119(D)	3	1(B)	123
	E	0	0	1	1
	P	1(C)	5	58(A)	64
	Total	120	8	60	188

Sensitivity = A / (A+B) = 58 / 59 = 98.3%

Specificity = D / (C+D) = 119 /120 = 99.2%

Accuracy = (A+D) / (A+B+C+D) = 177 / 179 = 98.9%

II. Precision:

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Sample	1	2	3	4
# Reps.	24	24	24	24
Mean (IU/ml)	291	126	52	8.0
S.D. (IU/ml)	15	12	3	0.3
C.V. (%)	5.2	9.4	5.6	4.2

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Sample	1	2	3	4
# Reps.	20	20	20	20
Mean (IU/ml)	257	109	56	8.6
S.D. (IU/ml)	11	8	5	0.6
C.V. (%)	4.4	7.4	8.7	7.2

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

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