GRUPO MEXLAB®

Chemiluminescence Immunoassay

Toxoplasma IgG

Summary of Assay Procedure

Step (20-25°C Room temp.)	Volume	Incubation time
Sample dilution 1:40 = 5 µL / 200 µL		
Diluted samples, controls & calibrators	100 µL	30 minutes
Washing buffer (3 times)	350 µL	
Enzyme conjugate	100 µL	30 minutes
Washing buffer (3 times)	350 µL	
Substrate A and Substrate B mixture	100 µL	5 minutes
Read with Luminometer in 5~30 minutes		

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

Toxoplasmosis is caused by the intracellular parasite Toxoplasma gondii and may be contracted by consuming contaminated meat or by 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 anomalies later in life. Toxoplasma IgG Chemiluminescence ELISA is an accurate serologic method to detect Toxoplasma 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 wells, and the Toxoplasma gondii IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1.Microwell strips: purified *Toxoplasma* antigen coated wells (12 x wells)

2.Sample diluent: Blue color solution. 1 vial (22 mL)

3.Washing concentrate 10x. 1 bottle (100 mL)

4.Enzyme conjugate: Red color solution. 1 vial (12 mL)

5.Substrate A: H_2O_2 in buffer. Natural bottle. 1 vial (6 mL)

6.Substrate B: Luminol in buffer. Amber bottle. 1 vial (6 mL)

7 .Negative Calibrator: 0 IU/mL. Natural Cap. 1 vial (150µL)

8.Cut-off Calibrator: 8 IU/mL. Yellow Cap. 1 vial (150µL) Toxo G Index = 1.0

9 .Positive Calibrator: 50 IU/mL. Red Cap. 1 vial (150µL)

10.Positive Calibrator: 150 IU/mL. Green Cap. 1 vial (150µL)

11.Negative control: Range on label. Blue Cap. 1 vial (150µL)

12.Positive control: Range on label. Brown Cap. 1 vial (150µL)

STORAGE AND STABILITY

1 .Store the kit at 2 - 8 °C.

2.Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.

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.

WARNINGS AND PRECAUTIONS

1.Potential biohazardous materials:The calibrator and controls contain human source components which have been tested and found nonreactive 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 in this kit are intended for use as a integral unit. The components of 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 HANDLING

1 .Collect blood specimens and separate the serum.

2.Specimens may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1.Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.

2.Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

1.Prepare 1:40 dilutions by adding 5 µL of the samples, negative control, positive control, and calibrators to 200 µL of sample diluent. Mix well. 2.Place the desired number of coated strips into the holder.

3.Dispense 100 µL of diluted sera, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

4.Remove liquid from all wells and repeat washing three times with washing buffer.

5.Dispense 100 µL of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

6.Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.

7.Mix equal volume of Substrate A & Substrate B, then dispense 100µL of this mixture to each well.

8.Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

Determination of Index values

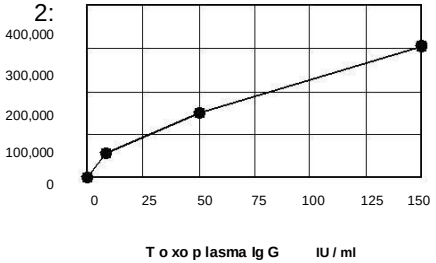
1 .Calculate the mean of duplicate RLU values (B).
2.Calculate the Toxo G Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C). Example 1:

Sample	Well No	RLU(A)	Mean RLU(B)	INDEX B/C
Cut-off Calibrator	A1 B1	134565 143585	139075 (C)	1
Positive C1 293197 296717 2.13 Calibrator D1 300238				
Negative E1 293197 30979 0.22 Control F1 300238				
Positive Control	G1 H1	554279	559332	4.02
Patient	A2	198463 564386	199761	1.43
Sample	B2	201059		

Quantitative determination of Toxoplasma IgG IU/ml value

For a quantitative determination of anti-Toxoplasma IgG levels of specimens in IU/ml unit, RLU of calibrators are plotted on Yaxis in graph versus their corresponding anti-Toxoplasma IgG concentration 0, 8, 50, and 150 IU/mL on X-axis. The estimates of levels in patient sera are read off the point to point curve using their individual RLU values.

Example 2:



QUALITY CONTROL

1.In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.
2.The RLU values vary with the different luminometer used. 3.Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

Negative: Toxo G Index of 0.90 or less are negative. Indicates absence of prior exposure to toxoplasma. (< 7 IU/mL)

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

Positive: Toxo G Index of 1.00 or greater or WHO IU/mL greater than 8 are seropositive. It indicates prior exposure to the toxoplasma virus.

If current infection is suspected, a second sample obtained 814 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.

Expected value and prevalence

48 random samples were determined with Toxo IgG CLIA Elisa. 5 were found to be positive. The positivity is 10%. Other set of 49 random samples were tested. The positivity is 6%. Prevalence may vary depending on a variety of the factors such as geographical location, age, socioeconomic status, race, type of the test employed, specimen collection and handling procedures, clinical and epidemiological history.

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity:

A total of 88 patient samples were used to evaluate specificity and sensitivity of the test. Toxoplasma test results were compared to a commercial ELISA kit results:

		Reference ELISA			
		N	E	P	Total
Toxoplasma IgG	N	56 (D)	0	2 (B)	58
Chemiluminescence ELISA	E	0	0	0	0
	P	0 (C)	0	30 (A)	30
	Total	56	0	32	88

Sensitivity = A / (A+B) = 30 / 32 = 94%

Specificity = D / (C+D) = 56 / 56 =100% Accuracy = (A+D) / (A+B+C+D) = 86 / 88 = 98%

Cross-reactivity

A study was performed to determine the cross-reactivity of Toxo IgG CLIA test with positive IgG samples. The results indicated an absence of cross-reactivity of the test: H. pylori, Rubella, CMV, HSV 1, HSV 2, Chlamydia trachomatis, EBV VCA, Measles, Mumps and VZV.

Precision

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	9.7%	7.8%	6.5%
Inter-assay	10.7%	8.5%	7.3%

LIMITATIONS OF THE PROCEDURE

1.Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
2.Toxoplasma antibody are present in apparently normal subjects in certain populations or geographic groups. A single test is not diagnostic for an active infection. Obtain two specimens at an interval of two weeks and test them at the same time to give more meaningful information.
3.As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

REFERENCES

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