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Sincerely,

Bio-Rad Laboratories

*Formerly National Committee for Clinical Laboratory Standards (NCCLS)

CLSI Toxoplasma IgG EIA
Cat. No. 25175

Procedure: For the qualitative, semi-quantitative or quantitative detection of human IgG antibodies to *Toxoplasma gondii* in human serum by enzyme immunoassay, as an aid in the determination of infection with Toxoplasma.

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE

Name and Intended Use

Name: Bio-Rad Toxoplasma IgG EIA.
Well color ID: Blue
Intended Use: For the qualitative, semi-quantitative or quantitative detection of human IgG antibodies to *Toxoplasma gondii* in human serum by enzyme immunoassay, as an aid in the determination of infection with *Toxoplasma*. When used as a qualitative test, Toxoplasma IgG EIA aids in the assessment of the patient's immunological response to *Toxoplasma*. These reagents have not received FDA clearance for use in testing blood or plasma donors.

Summary and Explanation of the Test

Serologic studies indicate that infection with *Toxoplasma gondii*, an intracellular parasite and the causative agent of toxoplasmosis, is fairly widespread in the population worldwide. For example, it has been estimated that 30% of the population in the United States exhibits serological evidence of exposure to *Toxoplasma gondii* (1). The organism can be transmitted during organ transplantation (2), by blood or leukocyte transfusion (3), contact with contaminated cat feces (4), or by ingestion of raw or undercooked meat from infected animals (5).

In adults the infection is usually asymptomatic, although symptomatic as well as fatal cases do occur. Symptoms range from swollen lymph nodes to those resembling infectious mononucleosis (1). In children,

the disease may affect the central nervous system and the viscera. Congenital infection also occurs, and toxoplasmosis is a significant cause of mortality and congenital malformation (6-9).

Specific IgG antibody titers directed against *Toxoplasma gondii* prior to pregnancy are correlated with immunity to infection (3). Inasmuch as infection may occur *in utero* if serologically negative women become infected during pregnancy, it is advisable for pregnant women to be tested for *Toxoplasma* specific antibodies early during their pregnancy, and serologically negative women should be monitored for *Toxoplasma* IgG antibody during their pregnancy and at delivery. Serologically positive results should be followed up by testing for *Toxoplasma* specific IgM in the newborn. Because less than one percent of newborns are born with maternally transferred IgM, the presence of *Toxoplasma* specific IgM antibodies is an indication of toxoplasmosis (10).

The results of serologic tests are of value as presumptive evidence of toxoplasmosis. The Toxoplasma IgG EIA test is intended for the detection of IgG antibodies to *Toxoplasma*. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, or as International Units (IU/mL), which are traceable to the WHO Anti-*Toxoplasma* Serum, 3rd International Standard Preparation, 1994.

Biological Principles of the Procedure

Diluted samples are incubated in antigen-coated wells. *Toxoplasma* antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme labeled antibodies to human IgG) is added and incubated. If IgG antibodies to *Toxoplasma* are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

SPECIMEN

Handling Conditions

Sera should be separated from clotted blood. If specimens are not tested within 8 hours, they should be stored at 2 to 8°C for up to 48 hours. Beyond 48 hours specimens should be stored at -20°C or below. Multiple freeze-thaw cycles should be avoided. Samples containing visible particulate matter should be clarified by centrifugation; and hemolyzed, icteric, or grossly contaminated samples should not be used. Samples should not be heat-inactivated before testing.

EQUIPMENT AND MATERIALS

Equipment and Materials Required But Not Provided

1. Microplate washer
2. Pipettors for dispensing 4, 100, and 200 µL
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm

Materials Provided

Toxoplasma IgG EIA
Product No. 25175 (96 Tests)
For *in vitro* diagnostic use.
Store these reagents at 2-8°C up to the expiration date indicated on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water. Do not remove desiccant

COMPONENT	CONTENTS	PREPARATION
Coated Wells 12 eight-well strips	• Coated with sonicated *Toxoplasma gondii* antigen, Strain: RH. • Blue wells	Use as supplied. Return unused strips to pouch and reseal. Do not remove desiccant.*
Well support 1 Frame	• Plate frame	Use as supplied.
Diluent** 1 bottle (25 mL)	• Phosphate-buffered saline with a protein stabilizer • Pink Color	Use as supplied.
Calibrator 1** 1 vial (0.5 mL)	• Human serum; Strongly reactive for *Toxoplasma* antibodies. • Index and IU/mL values shown on vial label.	Dilute in Diluent as described.
Calibrator 2** 1 vial (0.5 mL)	• Human serum; Moderately reactive for *Toxoplasma* antibodies. • Index and IU/mL values shown on vial label.	Dilute in Diluent as described.
Positive Control** 1 vial (0.5 mL)	• Human serum; Reactive for *Toxoplasma* antibodies. • Index and IU/mL values shown on vial label.	Dilute in Diluent as described.
Negative Control* 1 vial (0.5 mL)	• Human serum; Non-reactive for *Toxoplasma* antibodies.	Dilute in Diluent as described.
Conjugate** 2 bottles (12 mL)	• Goat anti-human IgG labeled with alkaline phosphatase (calf). • Green Color	Use as supplied.
Substrate** 1 bottle (12 mL)	• p-Nitrophenyl phosphate	Use as supplied.
Wash Concentrate** 1 bottle (30 mL)	• Tris-buffered saline • Tween 20™ • pH 8.0	Dilute in 1 liter of distilled or deionized water.
Stop Reagent 1 bottle (12 mL)	• Trisodium phosphate 0.5 M	Use as supplied.

* The color of the desiccant does not affect the performance of the kit.
** Contains 0.1% sodium azide.
*** The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

WARNINGS FOR USERS

1. For *in vitro* diagnostic use.
2. Test samples, Calibrator(s), Controls, and the materials that contact them should be handled as potential biohazards. The calibrators and controls have been tested and found to be non-reactive for HIV, hepatitis B surface antigen, and HCV antibodies by licensed tests. However, no method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent. Handle reagents and patient samples as if capable of transmitting infectious disease following recommended *Universal Precautions* for bloodborne pathogens as defined by OSHA (14), Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (15), WHO *Laboratory Biosafety Manual* (16), and/or local, regional, and national regulations.
3. The concentrations of anti-*Toxoplasma* in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
4. Avoid contact with open skin.
5. Never pipet by mouth.
6. Certain test reagents contain dilute **sodium azide** which may be harmful if enough is ingested (more than supplied in kit). Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. If disposing of solutions containing sodium azide down drains, flush with large volumes of water to minimize the build-up of metal-azide compounds. Dispose of contents and container in accordance with local, regional, national, and international regulations.
7. For more hazard information, refer to the product Safety Data Sheet (SDS), which is available at www.bio-rad.com and upon request.
8. Any lot number of the following reagents may be used with this assay, provided they have the correct catalog number and are not used beyond their labeled expiration date:
   - Diluent - Catalog # 25186
   - Substrate - Catalog # 25192
   - Wash Concentrate - Catalog # 25190
   - Stop Reagent - Catalog # 25191

   Do not mix any other reagents from different lots.
9. Do not use reagents beyond their stated expiration date.
10. Incubation times recommended in the Test Procedure section should be adhered to.
11. Unused Coated Wells should be kept in their resealable bag with desiccant and stored in the refrigerator.
12. This product should be used by qualified personnel.
13. There are no health hazards associated with the intact desiccant packet. Do not cut, split, or otherwise compromise it as dusts that may be generated could pose a health hazard. If the desiccant has been compromised, do not remove it from the plate pouch.

QUALITY CONTROL

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be ≥ 0.700, when read against the reagent blank.
3. The absorbance value of Calibrator 2 must be ≥ 0.300, when read against the reagent blank.
4. The absorbance value of the reagent blank should be < 0.350.

5. The Negative Control must have an Index value < 0.9 or an IU/mL value < 27.
6. The Positive Control must have an Index value ≥ 1.1, or an IU/mL value ≥ 33, and be within the range printed on the label. When performing qualitative tests, users may supply an alternative positive control if they wish.
7. To validate the upper range of the assay when performing the semi-quantitative and quantitative procedures, the Positive Control should be run at higher concentrations. For example, the Positive Control should be assayed at 1.5-fold and 2-fold concentrations by adding 6 µL and 8 µL of the Positive Control to 200 µL aliquots of the test set Diluent and transferring 100 µL of each of these dilutions to coated wells. The expected value ranges for these concentrated controls would be 1.5 times and 2 times, respectively, the expected value ranges printed on the Positive Control label. If the control values do not fall within the specified ranges, the assay is invalid and should be repeated. Optionally, users may supply alternative positive controls if they wish.
8. If any of these criteria are not met, the test is invalid and should be repeated. If the test is invalid, patient results can not be reported.

PROCEDURE

Procedure - Stepwise

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

1. Prepare working wash solution by adding entire bottle of Wash Concentrate (30 mL) to 1 liter of water. Once diluted, the wash solution can be stored at room temperature for up to two months, or at 4°C until the expiration date printed on the Wash Concentrate bottle.
2. Prepare 1:51 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the test set Diluent. For example: add 4 µL of sample to 200 µL of Diluent in a dilution well or tube and mix well.

   Note: For qualitative assays, a single Calibrator may be used; for semi-quantitative and quantitative assays, use Calibrator 1 and Calibrator 2.
3. Place an appropriate number of Coated Wells in the Well Support.

   Note: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.
4. Transfer 100 µL of each diluted Calibrator, Control and patient sample to the wells.

   Note: Include one well which contains 100 µL of Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results.
5. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.
6. Wash wells four times with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
7. Place 2 drops (or 100 µL) of Conjugate into each well.
8. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.
9. Wash wells four times with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
10. Place 2 drops (or 100 µL) of Substrate into each well.
11. Incubate at room temperature (20 to 25°C) for 30 ± 5 minutes.
12. Place 2 drops (or 100 µL) of Stop Reagent into each well. Tap the plate gently, or use other means to assure complete mixing.

13. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.

    Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

CALCULATION AND INTERPRETATION OF RESULTS

Calculation of Results

Qualitative results may be calculated using a single calibrator. For semi-quantitative and quantitative results, use a calibration curve consisting of two or more calibrators.

Single Calibrator (Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator 2 Index}}{\text{Calibrator Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve
Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point), and the reagent blank (zero/origin) using a point-to-point curve fit.

The upper range of the curve may be expanded by adding additional points. For example: The concentration of Calibrator 1 may be increased 1.5-fold and 2-fold by adding 6 µL and µL of Calibrator 1 to 200 µL of the test set Diluent and transferring 100 µL of each dilution to coated wells. The Index or IU/mL values assigned to these points should be 1.5 and 2 times, respectively, the value shown on the Calibrator 1 label. The extent to which the upper range of the standard curve may be expanded will be limited by the calibrator(s) being used.

Interpretation of Results

Index Value	IU/mL	Interpretation
< 0.9	< 27	Negative
≥ 0.9 and < 1.1	≥ 27 and < 33	Equivocal
≥ 1.1	≥ 33	Positive

The Toxoplasma IgG EIA cutoff values were based on statistical analyses, i.e., mean + 3 standard deviations, of 101 serum specimens shown to be negative by another legally marketed device. They were validated in tests of known positive and negative specimens (see Performance Characteristics).

When equivocal results are obtained, another specimen should be obtained two to three weeks later and tested in parallel with the initial specimen. If the second specimen is also equivocal, the patient is negative for primary or recent infection and equivocal for antibody status. If the second sample is positive, the patient can be considered to have a primary infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question is defined as seroconversion, and indicates active or recent infection.

To determine a significant difference between acute/convalescent serum pairs, both specimens should be assayed concurrently. Dose response experiments performed at Laboratory C (Miami, FL) have shown that a 90 to 110 percent increase in the Toxoplasma IgG EIA Index value corresponds to a two-fold increase in the *Toxoplasma* IgG antibody level; and a 180 to 220 percent increase in Toxoplasma IgG EIA Index value corresponds to a four-fold increase in the toxoplasma IgG antibody level.

Specimens which yield absorbance values above the range of the test set calibrator(s) may be pre-diluted in the test set Diluent and reassayed. The resulting Index value must be multiplied by the dilution factor. Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.

The suggested method for reporting results is: The following results were obtained with the Bio-Rad Toxoplasma IgG EIA test. Values obtained with different manufacturers' assay methods may not be used

interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer. When the assay is used qualitatively, the magnitude of results above the cutoff is not an indicator of total antibody present.

EXPECTED VALUES

The incidence of *Toxoplasma* IgG antibodies is related to age, socioeconomic condition and geographic location of the test population. In some areas 50% or more of the population at age 20 years show a positive serological test (12).

Serum samples obtained randomly from 143 normal South Florida blood donors were assayed by the Toxoplasma IgG EIA test. Forty-four samples (31%) were positive for IgG antibodies to *Toxoplasma*, ninety-six (67%) were negative, and three (2%) were equivocal. Of the positive samples, fifteen gave Index and IU/mL values greater than 7.5 and 226 respectively. The remaining twenty-nine positive samples yielded Index values between 1.2 and 7.5, and IU/mL values between 37 and 226. The mean Index and IU/mL values were 4 and 119, respectively. The ranges of these values are shown in Table 1.

Table 1. Results of tests of 143 specimens (100% frozen), from normal South Florida donors, performed at Laboratory C (Miami, FL), using the Toxoplasma IgG EIA test.

IU/mL Value Ranges	Index Value Ranges	Specimens	
< 30	< 1	97 {13}	67.8%
≥ 30 and < 50	≥ 1 and < 1.67	5 {1}	3.5%
≥ 50	≥ 1.67	41 {3}	28.7%

{ } Number of female donors of childbearing age.

Ninety-one women of childbearing age (18 to 45 years) were identified in the clinical studies. They ranged in age from 19 to 45, with a mean age of 32. Of these, 53 (58.2%) were positive, 2 (2.2%) were equivocal, and 36 (39.6%) were negative when tested by the Toxoplasma IgG EIA test. The ranges of values obtained for these women are shown in Table 2.

Table 2. Results of tests of 91 specimens, from women of childbearing age (18-45), performed at Laboratory A (Atlanta, GA), Laboratory B (Miami, FL) and at Laboratory C (Miami, FL), using the Toxoplasma IgG EIA test.

IU/mL Value Ranges	Index Value Ranges	Specimens	
< 30	< 1	38	41.8%
≥ 30 and < 50	≥ 1 and < 1.67	3	3.3%
≥ 50	≥ 1.67	50	54.9%

PERFORMANCE CHARACTERISTICS

Comparative Testing

Toxoplasma IgG EIA test results correlated very well with results of other serological tests. Sera from normal blood donors were assayed for the presence of *Toxoplasma* IgG antibodies, using the Toxoplasma IgG EIA test and three other commercial tests, at two independent laboratories (Lab A, Atlanta, GA, and Lab B, Miami, FL) and at Laboratory C (Miami, FL). These results are shown below in Tables 3, 4, and 5, respectively.

Table 3. Results of tests of 150 specimens (54% frozen and 46% fresh), from North and South Carolina, Alabama, Georgia, and Florida, performed at Laboratory A (Atlanta, GA), using the Toxoplasma IgG EIA test and another commercial test.

Comparative Test #1	Toxoplasma IgG EIA				
	Positive	Equivocal	Negative		95% CI**
Positive	111 {29}	0	1	Relative sensitivity	97.4 to 100%
Negative	2	4 {2}	32 {7}	Relative specificity*	86.2 to 100%
				Overall Agreement*	95.6 to 100%

*Excluding equivocal results
**Calculated by the Normal Method (13).
{ } Number of female donors of childbearing age.

Table 4. Results of tests of 153 specimens (77% frozen and 33% fresh), from South Florida, performed at Laboratory B (Miami, FL), using the Toxoplasma IgG EIA test and another commercial test.

Comparative Test #2	Toxoplasma IgG EIA				
	Positive	Equivocal	Negative		95% CI**
Positive	83 {20}	1	2	Relative sensitivity*	94.4 to 100%
Equivocal	0	0	2		
Negative	0	0	65 {16}	Relative specificity	95.5 to 100%
				Overall Agreement*	96.8 to 100%

*Excluding equivocal results
**Calculated by the Normal Method (13).
{ } Number of female donors of childbearing age.

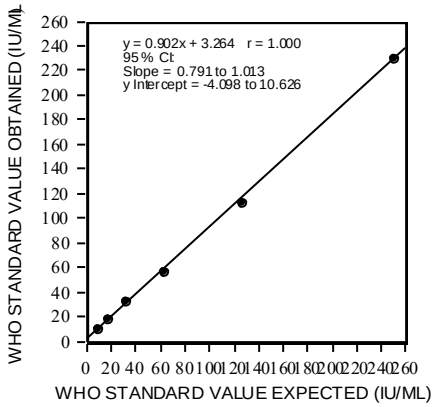
Table 5. Results of tests of 143 specimens (100% frozen), from South Florida, performed at Laboratory C (Miami, FL), using the Toxoplasma IgG EIA test and another commercial test.

Comparative Test #1	Toxoplasma IgG EIA				
	Positive	Equivocal	Negative		95% CI**
Positive	41 {3}	0	0	Relative sensitivity	93.0 to 100%
Equivocal	1	0	0		
Negative	3 {1}	2	96 {13}	Relative specificity*	93.6 to 100%
				Overall Agreement*	95.5 to 100%

*Excluding equivocal results
**Calculated by the Normal Method (13).
{ } Number of female donors of childbearing age.

The recovery of the WHO Anti-*Toxoplasma* Serum, using the Toxoplasma IgG EIA test, with the Toxoplasma IgG EIA secondary standard, is plotted below in Figure 1.

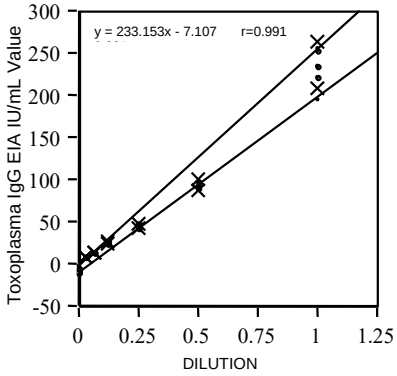
Figure 1. Recovery of the WHO Anti-*Toxoplasma* Serum, 3rd International Standard Preparation, Using the Toxoplasma IgG EIA Test.



Titration curve

Several strongly positive serum specimens were serially diluted (two-fold) in triplicate and assayed by the Toxoplasma IgG EIA test. Typical results are shown in Figure 2.

Figure 2. Titration Curve for a Strongly Positive Specimen.



The triplicate data for each dilution are shown as points, the 95% confidence limits for each set of triplicate data are indicated by (x's), and the 95% confidence limits for the slopes and y-intercepts are represented by straight lines. The formula for the linear regression for the triplicate data is shown in Figure 2.

Specificity

The Toxoplasma IgG EIA does not cross-react with IgG antibodies directed against the herpes viruses, which have been reported to cause heterotypic antibody responses. Of forty-five specimens which were unreactive in the Toxoplasma IgG EIA test, 19 were shown to contain moderate to high levels of antibody directed against cytomegalovirus, 24 against varicella zoster virus, 7 against Epstein-Barr virus, and 23 against herpes simplex virus types 1 & 2.

Precision

Eight serum specimens (2 negative and 6 positive) and the Toxoplasma IgG EIA positive and negative controls were assayed in triplicate on three separate occasions. The precision experiments were performed manually at two independent laboratories (Lab A and Lab B), and at Laboratory C. These results are shown below in Tables 6, 7, and 8, respectively.

Table 6. Results of intra-assay and inter-assay precision tests performed at Lab A. Values were calculated from Toxoplasma IgG EIA Index & IU/mL values.

Sample	Intra-Assay						Inter-Assay					
	Mean Index	S.D.	C.V.%	Mean IU/mL	S.D.	C.V.%	Mean Index	S.D.	C.V.%	Mean IU/mL	S.D.	C.V.%
Pos. Control	2.4	0.100	4.2	71.5	2.6	3.6	2.3	0.190	8.2	68.8	5.6	8.2
Neg. Control	0.6	0.000	NA	18.2	0.2	NA	0.6	0.050	NA	17.2	1.2	NA
1	0.7	0.058	NA	20.0	1.1	NA	0.6	0.071	NA	19.0	1.6	NA
2	0.8	0.000	NA	23.3	1.2	NA	0.7	0.078	NA	21.3	1.8	NA
3	2.8	0.289	10.2	85.0	7.4	8.7	2.6	0.397	15.0	79.4	11.3	14.3
4	2.4	0.153	6.5	70.5	5.3	7.5	2.2	0.224	10.3	65.0	6.4	9.8
5	5.0	0.600	12.0	151.0	18.0	11.9	5.0	0.546	11.0	149.8	16.3	10.9
6	3.4	0.586	17.1	102.6	17.8	17.4	3.2	0.557	17.2	96.7	17.2	17.8
7	2.0	0.058	2.8	61.2	3.0	4.9	2.2	0.219	10.1	65.1	7.0	10.7
8	2.1	0.100	4.8	62.8	2.6	4.1	2.5	0.450	17.8	75.9	13.4	17.6

Table 7. Results intra-assay and inter-assay precision tests performed at Lab B. Values were calculated from Toxoplasma IgG EIA Index & IU/mL values.

Sample	Inter-Assay						Inter-Assay					
	Mean Index	S.D.	C.V. %	Mean IU/mL	S.D.	C.V. %	Mean Index	S.D.	C.V. %	Mean IU/mL	S.D.	C.V. %
Pos. Control	1.9	0.217	11.6	56.2	6.6	11.7	1.9	0.140	7.3	57.4	4.2	7.3
Neg. Control	0.2	0.017	NA	4.8	0.4	NA	0.2	0.023	NA	5.1	0.7	NA
1	0.3	0.042	NA	8.0	1.2	NA	0.3	0.048	NA	8.7	1.3	NA
2	0.3	0.036	NA	9.3	1.1	NA	0.3	0.042	NA	10.0	1.4	NA
3	2.4	0.089	3.7	71.5	2.5	3.6	2.5	0.123	5.0	73.7	3.6	4.9
4	1.8	0.046	2.6	53.4	1.3	2.5	2.0	0.220	11.1	59.4	6.6	11.1
5	5.2	0.268	5.2	154.9	7.9	5.1	5.4	0.356	6.7	160.6	10.7	6.6
6	3.1	0.087	2.8	93.1	2.6	2.8	3.2	0.128	4.0	97.5	3.8	3.9
7	2.2	0.120	5.5	65.1	3.6	5.5	2.3	0.191	8.5	68.0	5.7	8.4
8	2.5	0.171	6.8	75.4	5.2	6.9	2.4	0.211	8.8	72.0	6.3	8.8

Table 8. Results intra-assay and inter-assay precision tests performed at Lab C. Values were calculated from Toxoplasma IgG EIA Index & IU/mL values.

Sample	Intra-Assay						Inter-Assay					
	Mean Index	S.D.	C.V.%	Mean IU/mL	S.D.	C.V.%	Mean Index	S.D.	C.V.%	Mean IU/mL	S.D.	C.V.%
Pos. Control	2.0	0.058	2.8	60.0	0.9	1.4	2.2	0.058	2.6	58.6	2.7	4.6
Neg. Control	0.2	0.000	NA	6.1	0.4	NA	0.2	0.000	NA	5.7	0.0	NA
1	0.3	0.000	NA	9.7	0.0	NA	0.3	0.033	NA	9.1	0.6	NA
2	0.4	0.058	NA	10.3	0.5	NA	0.3	0.044	NA	9.6	0.7	NA
3	2.4	0.115	4.7	72.1	3.6	5.0	2.3	0.183	8.0	67.8	5.2	7.6
4	1.9	0.200	10.5	56.3	5.9	10.5	1.8	0.206	11.5	53.9	5.7	10.5
5	5.1	0.586	11.6	150.8	18.2	12.1	4.8	0.412	8.6	143.1	12.6	8.8
6	3.0	0.058	1.9	88.3	2.4	2.7	2.8	0.158	5.6	84.0	5.1	6.1
7	2.1	0.100	4.8	62.9	2.9	4.5	2.0	0.176	8.8	59.9	4.6	7.8
8	2.2	0.058	2.6	67.2	0.8	1.1	2.3	0.078	3.4	68.7	2.4	3.5

LIMITATIONS OF THE PROCEDURE

The results obtained with the Toxoplasma IgG EIA test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

Paired specimens should be collected during the acute and convalescent stages of infection and tested concurrently to detect significant antibody increases. The semi-quantitative procedure should be used when testing paired sera only. Serum specimens obtained during the acute phase of infection may be negative by serological tests.

If the assay is used with cord blood as the specimen source, positive results should be interpreted with caution. The presence of IgG antibodies to *Toxoplasma* in cord blood may be the result of passive transfer of maternal antibody to the fetus. A negative result, however, may be helpful in ruling out infection. Performance characteristics have not been determined with neo-natal or cord blood.

The performance characteristics of the Toxoplasma IgG EIA test for any matrix other than serum have not been established.

Titration experiments (please see Figure 2) have shown that the upper limit of linearity for Toxoplasma IgG EIA IU/mL values is approximately 250.

PROCEDURE SUMMARY

1. Prepare working wash solution.
2. Prepare 1:51 dilutions of Calibrator(s), Controls and samples in the test set Diluent. Mix well.
3. Place 100 µL of the dilutions in the Coated Wells; reserve one well for the reagent blank.
4. Incubate at room temperature for 30 ± 5 minutes.
5. Drain wells thoroughly. Wash wells 4 times with Wash Solution and aspirate.
6. Place 2 drops (or 100 µL) of Conjugate in wells.
7. Incubate at room temperature for 30 ± 5 minutes.
8. Drain wells thoroughly. Wash wells 4 times with Wash Solution and aspirate.
9. Place 2 drops (or 100 µL) of Substrate in wells.
10. Incubate at room temperature for 30 ± 5 minutes.
11. Stop the enzyme reaction with 2 drops (or 100 µL) of Stop Reagent.
12. Read absorbance at 405 nm against reagent blank.

REFERENCES

1. Atlman, K.A., Toxoplasmosis, N.Y.State J. Med., 68(16):2151-2157, 1968.
2. Remington, J.S., Toxoplasmosis in the Adult, Bull. N.Y. Acad. Med., 50 (2):211-227, 1974.
3. McLeod, R. and Remington, J.S., in Harrison's Principles of Internal Medicine (Isselbacher et al eds), McGraw-Hill Book Co., 879-885, 1980.
4. Frenkel, J.K., *Toxoplasma* in and Around Us, Bioscience, 23:343-352, 1973.
5. Feldman, H.A., Epidemiology of *Toxoplasma* Infections, Epid. Rev., 4:204-213, 1982.
6. Walls, K.W. Serodiagnosis of Toxoplasmosis. Laboratory Management 16:26-31, 1973.
7. Remington, J.S. Toxoplasmosis, in *Obstetric and Prenatal Infections* (Charles, D. and Finland, M., eds). Lea & Febiger, Philadelphia, pages 27-74, 1973.
8. *A Procedural Guide to the Performance of the Serology of Toxoplasmosis*. Centers for Disease Control, Atlanta, 1976.
9. Lin, T.M., Halbert, S.P., and O'Connor, G.R. Standardized Quantitative Enzyme-Linked Immunoassay for Antibodies to *Toxoplasma gondii*., J. Clin. Microbiol. 11:675-681, 1980.
10. Walls, K.W., Serodiagnosis of Toxoplasmosis, Laboratory Management 26-31, January,1978.
11. Duncan, R.J.S., The Use of ELISA for Rapid Viral Dianosis: Antibody Detection. In ELISA *and Other Solid Phase Immunoassays*, D.M. Kemeny and S.J. Challacombe, eds. John Wiley & Sons, Ltd., Chichester.
12. Schaffer, J.G. and McQuay, R.M., Medical Protozoology, in Clinical Diagnosis by Laboratory Methods (Davidson, I. and Henry, J.B. eds.), W.B. Saunders Company, Philadelphia, 1969.
13. Gardner, M.J. and Altman, D.G., Confidence Intervals Rather Than Hypothesis Testing. Brit. Med. J., 292:746-750,1986.
14. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
15. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: US Government Printing Office, HHS Publication No. (CDC) 21-1112. December 2009.
16. World Health Organization. Laboratory Biosafety Manual. 3rd ed. Geneva: World Health Organization, 2004.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