

Laboratory Procedure Manual

Analyte: **Toxoplasma IgG antibodies**

Matrix: **Serum**

Method: **Enzyme immunoassay**

Method No.:

Revised:

as performed by: *Biology & Diagnostics, DPD, CID
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MODIFICATIONS/CHANGES: see *Procedure Change Log*

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab17_c	LBXTO1	<i>Toxoplasma</i> (IgG)

1. Summary of Test Principle and Clinical Relevance

The test procedure is a solid-phase enzyme immunoassay technique called an indirect enzyme immunoassay (EIA). Diluted test samples are placed into the *Toxoplasma gondii* antigen-coated wells of the microplate. Antibodies will bind to the *Toxoplasma* antigen. Unbound antibody and other serum proteins are removed by washing. Peroxidase-labeled monoclonal antibody specific for human γ -chain immunoglobulin (IgG) is added. The monoclonal antibody binds to the IgG antibody-*Toxoplasma* antigen-complexes attached to the microplate wells. Unbound conjugate is removed by washing. A solution of peroxidase substrate and chromogen tetramethylbenzidine (TMB) initiates a color reaction stopped by the addition of an acid. The enzymatic reaction, read as optical density on a spectrophotometer, is proportional to the quantity of *T. gondii* IgG antibody present in the test sample. The results are calculated using a standard curve and expressed as IU/mL.

Toxoplasmosis, caused by the parasite *T. gondii*, is usually an asymptomatic infection with few serious after-effects. However, people with acquired immune deficiency syndrome (AIDS) may develop life-threatening central nervous system disease. Infection during pregnancy may also cause severe congenital abnormalities. Because parasitic infection is very difficult to diagnose, the detection of *Toxoplasma*-specific antibody is an acceptable means of confirming infection (1–4).

2. Safety Precautions

Wear gloves, lab coat, and safety glasses while handling all human blood products. Place disposable plastic, glass, and paper (pipette tips, gloves, microtiter plates, etc.) that contact serum samples in a hazardous waste container prior to autoclaving. Wipe down all work surfaces with 10% sodium hypochlorite (bleach) solution when work is finished.

Material safety data sheets (MSDSs) for sulfuric acid, tetramethylbenzidine, and sodium hypochlorite are kept in the *Working Safely with Hazardous Chemicals* manual.

3. Computerization; Data System Management

A. The integrity of specimen and analytical data generated by this method is maintained by computer-assembled data and data stored in multiple computer systems. Specimen ID files for each shipment are e-mailed by NHANES to the lab and stored on the Division network. Data files containing the date, run ID, and specimen test results by specimen ID are stored on the Division network and a Zip disk. The completed data files are routinely sent to the NHANES data manager in Maryland by e-mail. Specimen tracking is accomplished by merging all *Toxoplasma* files in an Excel file on the Division network.

B. Routine backup procedures include nightly archive of the network drive.

C. Documentation for system maintenance is contained in hard copies of data records for 5 years.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

A. No special instructions such as fasting or special diets are required.

B. Specimen type: may be serum or plasma.

C. The optimal amount of specimen required is 0.5 mL to allow for repeat testing; the minimum volume is 20 μ L (0.02 mL).

D. Specimen stability has been demonstrated for 5 years at $\leq -20^{\circ}\text{C}$.

- E. The criterion for unacceptable specimen volume is <0.02 mL.
 - F. Hyperlipemic or hemolyzed serum is not used.
 - G. Specimen handling conditions require separation of serum/plasma from cells and storage of the specimen at $\leq -20^{\circ}\text{C}$ until time of analysis. Samples thawed and refrozen are not compromised.
5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides
- Not applicable for this procedure.
6. Equipment and Instrumentation Materials, Reagent Preparation, Calibrators (Standards), and Controls
- A. Instrumentation
 - (1) Vmax microplate reader with computer equipped with SOFTmax PRO software for reader control and data analysis and printer (Molecular Devices Corp., Menlo Park, CA).
 - (2) Pipette, 12-channel variable-volume, 50 to 200 μL (Rainin, Woburn, MA).
 - (3) Pipette, variable-volume, 1 to 10 μL (Rainin, Woburn, MA).
 - (4) Water bath, 37°C (Fisher Scientific, Atlanta, GA).
 - (5) Vortex mixer (Fisher Scientific, Atlanta, GA).
 - (6) Magnetic stirrer and stir bars (Fisher Scientific, Atlanta, GA).
 - B. Other Materials
 - (1) Platelia Toxo IgG TMB kit (Bio-Rad Laboratories, Hercules, CA).
 - (2) Deionized water (Millipore, Bedford, MA).
 - (3) 25-, 50-, 100-, and 1000-mL graduated cylinders (Corning Glass Works, Corning, NY).
 - (4) 50-mL plastic beaker for preparing working substrate (any vendor).
 - (5) 1000-mL screw-cap glass Wheaton bottle (Wheaton Industries, Millville, NJ).
 - (6) 96 Titertube micro tubes, racked (Bio-Rad Laboratories, Hercules, CA).
 - (7) Pipette tips (1 to 200 μL) for multichannel pipette, racked (Rainin, Woburn, MA).
 - (8) Pipette tips (1 to 10 μL) (Rainin, Woburn, MA).
 - (9) Kay-Dry paper towels (Kimberly-Clark Corp., Roswell, GA).
 - (10) Vinyl examination gloves (any vendor).
 - (11) 10% sodium hypochlorite (bleach) solution (any vendor).
 - (12) 1.0-mL disposable plastic pipettes (any vendor).
 - (13) Test tube racks, 13 mm, for specimen vials (any vendor).

C. Reagents and Reagent Preparation

All reagents are supplied in the Platelia Toxo IgG TMB kit. All reagents are stored at 4°C–8°C.

- (1) Inactivated *T. gondii* antigen (RH strain)
Coated on wells in microplate tray containing 12 strips with 8 breakable wells each. Allow strips to reach 20°C–25°C before opening sealed bag. Remove only the required number of strips. Unused strips can be stored up to 1 month at 2–8°C if bag is tightly sealed.
- (2) Wash solution concentrate (10×), 100 mL
Tris sodium chloride buffer (pH 7.4), 1% Tween-20 (polyoxyethylene-20 sorbitan monolaurate), and 0.01% thimerosal (C₉H₉HgO₂SNa). Dilute to working solution with deionized water.
- (3) Conjugate concentrate (50×), 0.6 mL
Murine monoclonal antibody to human γ chains coupled with horseradish peroxidase. Dilute with sample diluent.
- (4) Sample and conjugate diluent
2 80-mL bottles, each containing tris-sodium chloride buffer (pH 7.7), bovine serum albumin (BSA), phenol red, 0.1% Tween-20, and 0.01% thimerosal.
- (5) Chromogen
Solution containing tetramethylbenzidine (TMB).
- (6) Substrate buffer
60 mL containing (pH 4.0) citric acid and sodium acetate, 0.015% (v/v) hydrogen peroxide, and 4% Me₂SO (DMSO).
- (7) Stopping solution
28 mL; 1 N H₂SO₄. **CAUTION: CORROSIVE.**
- (8) Working washing solution (WWS)
Dilute 100 mL of washing solution concentrate (WSC) in 900 mL of deionized water for each microtiter plate. Mix thoroughly. Store the diluted wash solution at 2–8°C for a maximum of 14 days.
- (9) Working conjugate (WC)
- (10) Dilute 0.5 mL of 50× conjugate concentrate in 25 mL of sample/conjugate diluent for each plate 15 min before the end of the first incubation period. Mix thoroughly.
- (11) Substrate-chromogen solution (SCS)
Add 1.0 mL of chromogen to each 10 mL of substrate buffer 5 min prior to the end of the second incubation period. Protect solution from light until ready to use. Once prepared, use within 6 hours.

D. Standards Preparation

Calibration standards containing human serum, bovine serum albumin, glycerol, 0.01% thimerosal, and 0.5% Proclin 300 are supplied ready to use as 0-, 6-, 60-, and 240-IU/mL. The 0 IU calibrator contains human serum non-reactive for *T. gondii* IgG antibodies. The 6-, 60-, and 240-IU calibrators contain human serum reactive for *T. gondii* IgG antibodies.

The standards are calibrated by Bio-Rad Laboratories to the World Health Organization's "TOXM 185" standard serum in International Units (IU).

E. Preparation of Quality Control (QC) Materials

In-house QC samples from previously analyzed human serum samples are selected for reactivity in the low concentration ranges of the curve. The QC samples are then separated into appropriately small aliquots to minimize any influence of repeat freezing and thawing. Samples are coded and stored at $\leq -20^{\circ}\text{C}$, and are considered to be stable indefinitely.

Sufficient material is separated into aliquots to ensure a supply of QC material sufficient to last the length of the NHANES IV project.

7. Calibration and Calibration Verification Procedures

A. Calibration Curve

- (1) A calibration curve is constructed by using the measured absorbance value of the 0-, 6-, 60-, and 240-IU standards plotted versus concentration. The 4-parameter curve fit should be chosen in Softmax software.
- (2) If the calibration curve correlation coefficient is >0.98 , the test is acceptable, and test results may be printed.

B. Verification

- (1) To validate the assay, the manufacturer states that the following criteria must be met:
 - (a) $\text{OD A1} \leq 0.2$
 - (b) $\frac{\text{OD B1}}{\text{OD A1}} > 2.0$
 - (c) $\text{OD D1} > 1.0$

Where A1, B1, and D1 refer to microtiter plate wells containing the 0-, 6-, and 240-IU standards, respectively, and OD represents the optical density.

- (2) The two QC samples in positions A6 and A7 should be > 10 IU but < 50 IU.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

- (1) Place the specimens in test tube racks in following positions:
 - (a) Calibrators in A1, B1, C1, D1, and E1.
 - (b) QC specimens in F1 and G1.
 - (c) Unknowns 1-89 in A2–H12.
- (2) Bring reagents and serum specimens to 20°C – 25°C before use.
- (3) Prepare the working washing solution (WWS).
- (4) Label the microwell strips A–H according to their position in the plate holder.

B. Sample preparation

- (1) Vortex each sample.
- (2) Dilute the calibrators, patient specimens and QC controls 1:101 by adding 5 μ L of sample to 500 μ L of diluent in the appropriate tube of the racked microtubes.

C. Instrument setup for the Vmax plate reader.

- (1) Turn on both the computer and the Vmax reader 10 minutes before needed.
- (2) Choose SOFTMAX from the PC main menu, then choose ASSAYS, then ASSAYS, then TOXOG protocol. The following parameters are automatically set by the TOXOG protocol:
 - Mode: Endpoint L1–L2
 - Wavelength L1: 450
 - Wavelength L2: 650
 - Data display: Analyzed
 - Std curve: 4-parameter
 - Automix, Autocal, Autodisk, Autoprint: ON

D. Operation of Assay Procedure

- (1) Wash microplate strips once with WWS. Invert the microplate and gently tap on the absorbent paper to remove remaining liquid.
- (2) With the multichannel pipette, add 200 μ L of diluted samples to the appropriate wells corresponding to the sample position. Cover the microplate with adhesive film.
- (3) Incubate the microplate at $37 \pm 2^\circ\text{C}$ in the water bath for 60 ± 5 min.
- (4) Prepare the working conjugate (WC) 15 min before the end of the first incubation period.
- (5) Remove the adhesive film and discard well contents into container with 10% bleach. Wash the microplate three times using WWS. Invert the microplate and gently tap on the absorbent paper to remove remaining liquid.
- (6) Add 200 μ L of conjugate solution to each well with the multichannel pipette. Cover the microplate with adhesive film.
- (7) Incubate the microplate at $37 \pm 2^\circ\text{C}$ for 60 ± 5 min.
- (8) Prepare substrate-chromogen solution 5 min prior to the end of the second incubation period. Protect from light until ready to use.
- (9) Remove the adhesive film and discard well contents. Wash the microplate four times using WWS. Invert the microplate and gently tap on the absorbent paper to remove remaining liquid.
- (10) Add 200 μ L of substrate-chromogen solution to each well with the multichannel pipette.
- (11) Incubate the microplate in the dark at $20\text{--}25^\circ\text{C}$ for 30 ± 2 min.
- (12) Add 100 μ L of stopping solution to each well with the multichannel pipette.
- (13) Wipe the underside of plate wells dry with a lint-free tissue to remove condensation.

- (14) Read the microtiter plate in the Vmax.
- (15) Perform the validation calculations, save the data, and print the report.
- (16) Export the data as a text file, save in k: m_109_1304_sftmax as GMMDDYY.txt
- (17) Open the .txt in Excel and convert. Merge the values with the appropriate specimen ID and save.
- (18) Convert the merged file to a .csv file and e-mail to the NHANES data manager.

E. Recording of Data

(1) Quality Control Data

For each run, enter the following on the master sheet in the NHANES IV binder: kit lot number, expiration date, plate ID, IU values of both QC samples, the absorbance value of B1 divided by the absorbance value of A1, and the absorbance value of D1.

(2) Analytical Results

The raw and analyzed data are printed automatically under the SOFTmax program. The printed report should be filed in the NHANES IV data binder. The results are merged with the IDs as explained in sections 8.d.16-17 and saved in the NHANES Master.xls

F. Replacement and Periodic Maintenance of Key Components

- (1) Vmax lamp: a spare lamp should be available. Order another if the spare is used for replacement.
- (2) All micropipettes used in testing clinical specimens should be checked for calibration every 6 months. Pipettes that do not conform to specifications should be autoclaved and sent out for recalibration in accordance with manufacturer's recommendations. Calibration records should be kept for each pipette by serial number.

G. Calculations

The SOFTmax program uses a 4-parameter curve fit to calculate the calibration curve. The unknown value is read off the curve in IU. The SOFTmax program generates a plotted curve and provides curve statistics and correlation coefficients. The correlation coefficient should be ≥ 0.975 .

H. Procedure Notes

The substrate-chromogen solution should be colorless to very pale yellow. Discard it if it is yellow-orange.

9. Reportable Range of Results

- A. Samples with results < 10 IU are reported as non-reactive for the presence of antibodies to *Toxoplasma*.
- B. Samples with optical density readings ≥ 10 IU are reported reactive for the presence of *T. gondii*.
- C. Results > 240 IU are reported as "240."

10. QC Procedures

The method described in this protocol has been used for 10 years for *in vitro* diagnostic testing. The method has proven to be accurate, precise, and reliable. The calibration standards used in this assay are

provided by Bio-Rad Laboratories and are calibrated to the World Health Organization's "TOXM 185" standard serum. Estimates of imprecision can be generated from long-term QC results.

"Bench" QC specimens are used in this quantitative method. The control specimens are inserted by the analyst in each plate so that judgments may be made on the day of analysis. Results are obtained by running these samples through the complete testing procedure. The data from these materials are used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

Each assay includes blinded in-house controls containing low levels of antibodies to *Toxoplasma*. These controls, which are run in duplicate, are randomly scattered throughout the assay. The in-house controls are prepared in sufficient quantity to provide serum samples for all runs for the length of NHANES IV project. The pools are aliquoted into small vials and stored at $\leq -20^{\circ}\text{C}$. *Toxoplasma*-specific serum antibodies will remain stable indefinitely when stored in this fashion.

QC limits are established for each pool. An analysis of variance is performed for each pool after 30 or more characterization runs have been performed.

Table 1 shows the precision and accuracy of a representative pool used for NHANES III.

Table 1. Precision and Accuracy

n	Mean	95% limits	99% Limits	Runs	Total CV
62	14.95	14.2–15.7	14.0–15.9	31	19.26

Control samples are placed in each microtiter plate in wells F1 and G1. After the results of the QC specimens are determined, the long-term QC charts are examined to determine if the system is "in control."

11. Remedial Action if Calibration or Qc Systems Fail to Meet Acceptable Criteria

Consult with the supervisor for appropriate corrective actions. Check to see that plate reader is operating properly. If necessary, reanalyze all specimens.

12. Limitations of Method; Interfering Substances and Conditions

Do not use hyperlipemic or hemolyzed serum.

13. Reference Ranges (Normal Values)

The prevalence of chronic toxoplasmosis among adults will vary significantly in different populations.

The kit manufacturer recommends that all titers of <10 IU be considered non-reactive and all titers ≥ 10 IU be considered reactive. A positive result is an indication of infection with *T. gondii*.

14. Critical Call Results (Panic Values)

Not applicable.

15. Specimen Storage and Handling During Testing

Allow specimens to reach and maintain 20–25°C during analysis. Otherwise, store them at $\leq -20^{\circ}\text{C}$.

16. Alternative Methods for Performing Test or Storing Specimens if Test System Fails

This laboratory does not have an alternative method for performing this test. Specimens will be stored at $\leq -20^{\circ}\text{C}$ until testing resumes.

17. Test Result Reporting System; Protocol For Reporting Critical Calls (If Applicable)

Not applicable.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Standard record-keeping means (e.g., electronic Excel, mainframe data files, floppy disc) should be used to track specimens. The records will be maintained for 5 years, and duplicate records will be kept off-site in electronic format. Excess serum is stored in NCHS, CDC.

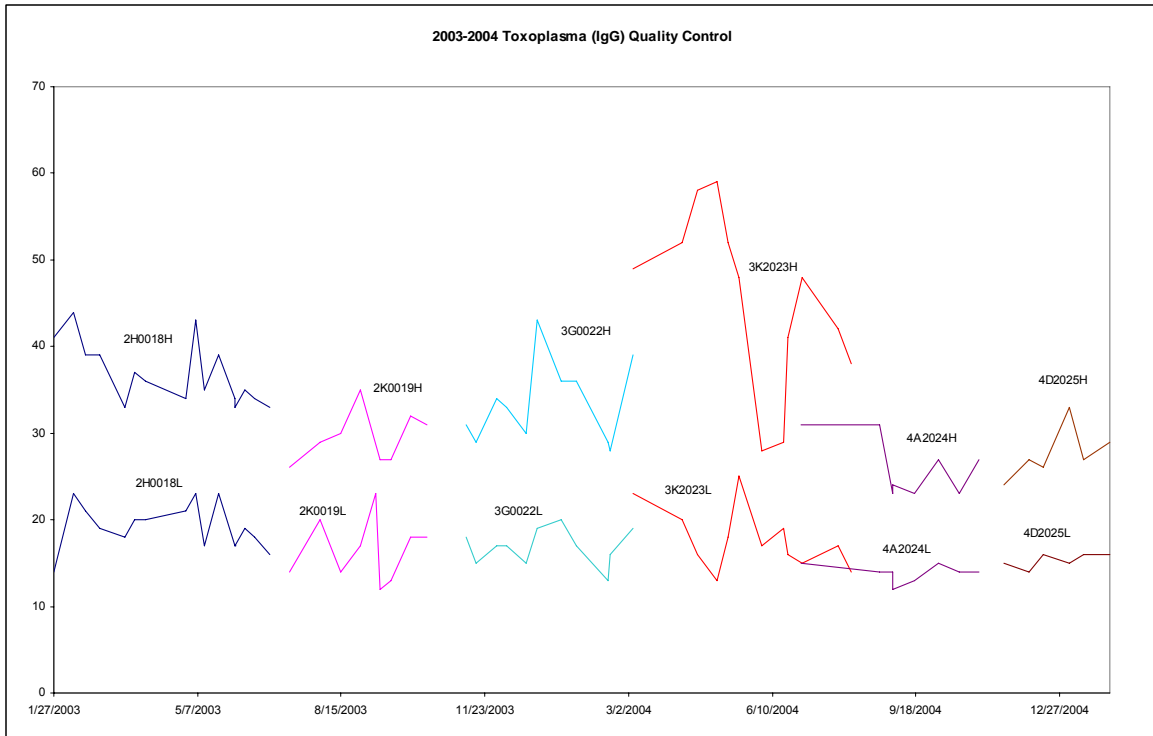
19. Summary Statistics and QC Graphs

A. Toxoplasma (IgG)

Summary Statistics for Toxoplasma (IgG) by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2H0018	16	1/27/2003	6/26/2003	19.1	2.7	13.9
2H0018	16	1/27/2003	6/26/2003	36.8	3.6	9.8
2K0019	9	7/10/2003	10/14/2003	16.6	3.6	21.8
2K0019	8	7/10/2003	10/14/2003	29.6	3.0	10.2
3G0022	11	11/10/2003	3/5/2004	16.9	2.1	12.3
3G0022	11	11/10/2003	3/5/2004	33.5	4.7	14.1
3K2023	12	3/5/2004	8/4/2004	17.8	3.5	20.0
3K2023	12	3/5/2004	8/4/2004	45.3	10.1	22.2
4A2024	8	6/30/2004	11/1/2004	13.9	1.0	7.1
4A2024	8	6/30/2004	11/1/2004	26.1	3.4	13.2
4D2025	6	11/18/2004	1/31/2005	15.3	0.8	5.3
4D2025	6	11/18/2004	1/31/2005	27.7	3.1	11.1

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