

Laboratory Procedure Manual

Analyte: **Toxoplasma IgM Antibodies**

Matrix: **Serum**

Method: **Enzyme Immunoassay**

Method No.:

Revised:

as performed by: **Biology & Diagnostics, DPD, CID
Division of Laboratory Sciences
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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	LBXTO2	<i>Toxoplasma (IgM)</i>

1. Test Principle and Clinical Relevance

Toxoplasmosis, caused by the parasite *Toxoplasma gondii*, is usually an asymptomatic infection with few serious after-effects. However, patients with acquired immune deficiency syndrome (AIDS) may develop life-threatening central nervous system disease. Also, infection during pregnancy may cause severe congenital abnormalities. Parasitological diagnosis of infection is very difficult, so detection of Toxoplasma-specific antibody is the acceptable means of confirmation of infection. The test procedure is an IgM antibody-capture enzyme immunoassay (EIA).

2. 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: serum or plasma separated from cells.
- C. Optimal amount of specimen required is 2.0 ml; the minimum is 50 μ l (0.05 ml).
- D. Specimen stability has been demonstrated for 5 years at -20°C .
- E. The criteria for unacceptable specimen is a low volume (<0.02 ml). An additional specimen will be requested through DASH.
- F. Contaminated or hyperlipemic serum may compromise test results.
- G. Specimen handling conditions: serum/plasma should be separated from cells, shipped at room temperature, and stored at -20°C until analysis. Thawed and refrozen samples are not compromised.
- H. Special safety precautions: Wear gloves, lab coat, and safety glasses while handling all human blood products. Disposable plastic, glass, and paper (pipette tips, gloves, microtiter plates, etc.) that contact patient samples are to be placed in an appropriate covered container prior to autoclaving. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished. Avoid skin and mucous membrane contact with the substrate buffer, chromogen (O-phenylene diamine [OPD]), and stopping solution due to a risk of toxicity, irritation, burns, and chronic effects. All material safety data sheets (MSDS) for hazardous chemicals are available in the lab manual "Working Safely with Hazardous Chemicals".

3. Reagents, Supplies and Equipment

- A. Chemicals/reagents
 - (1) *T. gondii*-soluble tachyzoite antigen, peroxidase-labeled (CDC).
 - (2) Anti-human IgM (goat), affinity-purified (BioSource).
 - (3) $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
 - (4) $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$
 - (5) NaCl
 - (6) Tween-20
 - (7) Deionized water (dH_2O)
 - (8) Bleach (10% sodium hypochlorite solution)
 - (9) O-phenylene diamine (OPD), Kodak lot B9A
 - (10) $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$
 - (11) Acetic acid
 - (12) Methanol

- (13) 30% H₂O₂ (Baker ULTREX)
- (14) concentrated H₂SO₄ (18 M)
- (15) Bovine serum albumin (BSA), Fraction V, lot 112F-9390 (Sigma).
- (16) Serum calibrators: Controls:
 - Negative - Boyte
 - High positive - JD 1/24/78
 - Negative -
 - Low positive - Nor 2/6/78

B. Supplies

- (1) 25 ml graduated cylinder.
- (2) 50 ml graduated cylinder.
- (3) 1000 ml graduated cylinder.
- (4) 500 ml squeeze bottle.
- (5) 1 ml syringe with egg needle.
- (6) Immulon II microtiter well strips or plates (Dynatech Laboratories).
- (7) Pressure-sensitive film for microtiter plates (Becton Dickinson)
- (8) Pipette tips (1 µl–200 µl) for Titertek, racked (Marsh).
- (9) Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark).
- (10) Vinyl examination gloves (Travenol).
- (11) Test tube racks, 16 mm, for specimen vials (Nalgene).
- (12) Plastic microtubes, racked in 96-well format (Bio-Rad).

C. Instrumentation

- (1) vMax microplate reader with IBM computer equipped with SOFTmax software for reader control and data analysis, and printer (Molecular Devices Corp., Menlo Park, CA).
- (2) Vortex-Genie vortex mixer (Fisher).
- (3) Magnetic plate stirrer (Fisher).
- (4) 12-channel variable volume 50 µl–250 µl Titertek pipette (Labsystems).
- (5) 12-channel variable volume 5 µl–50 µl Titertek pipette.
- (6) EDP2 25 µl and 250 µl pipettes (Rainin).
- (7) Water bath, 37°C.

D. Preparation

- (1) Phosphate buffer saline, 0.01M (PBS)
- (2) Prepare 2 solutions as follows:
 - Solution A: 1.3799 g NaH₂PO₄·H₂O + 8.766 g NaCl, qs to 1 liter with dH₂O
 - Solution B: 2.6807 g Na₂HPO₄·7H₂O + 8.766 g NaCl, qs to 1 liter with dH₂O

Combine and mix solutions A and B (approximately 5 parts of A:6 parts of B) until the pH is 7.2. Autoclave to sterilize.
- (3) Anti-µ (BioSource anti-µ usually titers at 1:100)
For one plate: 9.9 ml PBS + 0.1 ml anti-µ
- (4) 0.01 M PBS, pH 7.2 / 0.05% Tween-20 (PBS/T)
1 liter PBS + 0.5 ml Tween-20
- (5) 0.01 M PBS, pH 7.2 / 0.05% Tween-20 / 1% BSA (PBS/T/BSA)

500 µl per specimen are needed:

No. of strips:		1	2	3	4	6	8
PBS/T	ml	4	7	10	13	20	26
BSA	mg	40	70	100	130	200	260

(6) Toxoplasma antigen (1:100) :

No. of strips:		1	2	3	4	6	8
PBS/T	ml	1.5	3.0	4.0	5.5	8.0	9.9

(7) OPD Substrate

0.05 M NaAc buffer: 6.8 g NaC₂H₃O₂·3H₂O in 1 liter dH₂O
 Adjust pH to 4.5 with acetic acid.

Stock OPD: 3.0 ml methanol + 30 mg OPD powder.

Working OPD solution: 10.0 ml NaAc buffer
 0.1 ml stock OPD
 10 µl 30% H₂O₂

(8) 4 M H₂SO₄

22.2 ml concentrated (18 M) H₂SO₄
 77.8 ml H₂O

E. Performance Parameters

The absorbance values of the calibrators should be:

Boyte.....0.000–0.500
 JD 1/24/78.....>1.0

F. Storage requirements

- (1) The antigen-sensitized plates must be stored at 4°C. Expiration date is 12 months post sensitization.
- (2) The stock buffers may be stored in a glass screw cap bottle at 4°C until depleted.

4. Quality Control (QC)

The toxoplasma EIA-IgM test is considered in control provided:

- A. The negative control is < 2.0
- B. The low positive control is ≥ 2.0 but < 4.0.

5. Procedure

A. Preliminaries

- (1) Add 100 µl of anti-µ per well to Immulon II microtiter plates with a 12-channel Titertec pipette. Seal, incubate for 2 hours floating in the 37°C water bath, and then store plates at 4°C until needed.

- (2) Place samples to be tested in test tube rack. Record the specimen position on a toxoplasma EIA run sheet; test all calibrators, controls, and unknown samples in duplicate.
- (3) Bring reagents and serum specimens to room temperature (15–25°C) before use.
- (4) Determine the number of antigen-sensitized wells needed; place strips in rack and label.
- (5) Prepare PBS/T diluent.
- (6) Prepare PBS/T/BSA diluent.

B. Sample preparation

- (1) Vortex each sample.
- (2) Add 495 µl (250 +245) of PBS/BSA/T with the 12-channel Titertec pipette to all appropriate microtubes.
- (3) Add 5 µl of patient specimen to appropriate microtube.

C. Test procedure

- (1) Wash microplate by shaking out contents and by filling and emptying wells 3 times with PBS/T with the squeeze bottle. Invert plate and gently tap on absorbent paper to remove remaining liquid.
- (2) Using the multichannel pipette, mix specimen dilutions. Transfer 100 µl of specimen dilution to each of two wells.
- (3) Cover microplate and incubate at 37°C in water bath for 30 minutes.
- (4) Wash microplate by shaking out contents, filling wells with PBS/T and soaking for 3 min. Repeat for a total of 3 times. Invert microplate and gently tap on absorbent paper to remove remaining liquid.
- (5) Add 100 µl of diluted conjugated toxoplasma antigen to each well with the 12-channel Titertek pipette.
- (6) Cover microplate and incubate microplate at 37°C in water bath for 30 minutes.
- (7) Wash microplate as in Step (5) above.
- (8) Add 100 µl of working substrate solution to each well with the 12-channel Titertek pipette and mix by tapping plate. Incubate at room temperature in the dark for 30 minutes.
- (9) Add 25 µl of 4N H₂SO₄ to each well with the Titertek and mix by tapping.
- (10) Wipe the bottom of wells dry with a lint-free tissue.

D. Instrument setup for the Vmax plate reader.

- (1) Turn on both the computer and the Vmax reader 10 minutes prior to reading.
- (2) Choose the SoftMax icon in the Windows menu, Toxoplasma IgM Assay (reads at 490 I).
- (3) Read the microtiter plate and print the raw data.
- (4) Blank on Boyte and print the adjusted data.
- (5) Calculate:

$$\text{Result} = 10 \times \frac{\text{mean abs. of unknown}}{\text{mean abs. of JD 1/24}}$$

E. Recording of Data

- (1) QC Data. For each run, enter the following on the Toxoplasma IgM EIA master sheet in the Quality Control binder: anti-IgM lot number, sensitization date, antigen lot, run date, OD values of Boyte and JD 1/24 calibrators, and result of Negative and Nor 2/6 controls.

- (2) Analytical Results. Any result above 2.0 is considered positive. The EIA-IgM result should be placed in the appropriate place on the Toxoplasma IFA run sheet. The run template should be pasted to the printed data sheet and filed in the Toxoplasma IgM data binder.

4. Reporting and Interpreting Results

A. Reference ranges

The prevalence of Toxoplasma will vary significantly in different populations.

All EIA-IgM reactions ≥ 2.0 are considered POSITIVE, indicating infection with *T. gondii* at some point in time, probably within the last 6 months. Sensitivity was 100% and specificity was 99.1% as compared to the Remington DS-EIA-IgM assay (see Appendix B).

B. Procedures for abnormal results

Report as the actual result.

C. Reporting format

If IFA-IgG is NEGATIVE and EIA-IgM is NEGATIVE, there is no evidence of exposure.

If IFA-IgG is POSITIVE and EIA-IgM is:

- (1) NEGATIVE, then infection was probably acquired more than 1 year ago.
- (2) ≥ 2.0 , it is probably a recent infection acquired within the past 6 months.

5. Procedure Notes

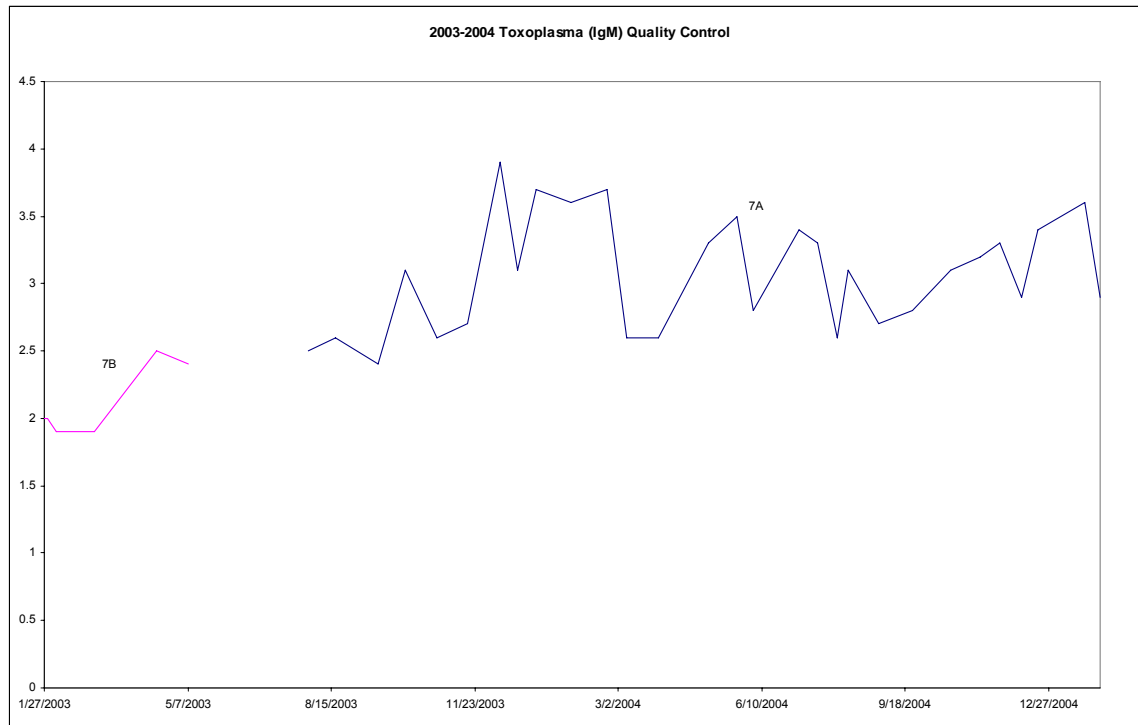
Remedial action is required if calibration or QC systems fail to meet acceptable criteria.

6. Summary Statistics and QC Graphs

A. Toxoplasma (IgM)

Summary Statistics for Toxoplasma (IgM) by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
7B	6	1/27/2003	5/7/2003	2.12	0.26	12.5
7A	29	7/30/2003	2/1/2005	3.07	0.42	13.7



References

1. Wilson, M, Remington, JS, Clavet, C, Varney, G, Press, C, Ware, D, et al. Evaluation of six commercial kits for detection of human IgM antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. 1997;35:3112–3115.
2. Wilson, M, McAuley, JM. Manual of Clinical Microbiology, 7th ed. Murray, PR, Baron, EJ, Pfaller, MA, Tenover, FC, Tenover, FC, Tenover, RH, editors. Washington, DC: American Society for Microbiology;1999, 1374–1382.
3. Remington JS, McLeod, R, Desmonts, G. Infectious Diseases of the Fetus and Newborn Infant, 4th ed. Remington JS, Klein, JO, editors. Philadelphia: W.B. Saunders Co; 1995, 140–266.