Laboratory Procedure Manual

Analyte: Toxoplasma IgM
Matrix: Serum
Revised: October 25, 2004

as performed by: Biology & Diagnostics, DPD, CID
Division of Laboratory Sciences
National Center for Environmental Health

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Important Information for Users
CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.
Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001–2002 data.

A tabular list of the released analytes follows:

<table>
<thead>
<tr>
<th>Dataset name</th>
<th>Variable name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>L17_b</td>
<td>LBXT01</td>
<td>Toxoplamsa IgM</td>
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</table>

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 µl (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) NaH₂PO₄·H₂O
   (4) Na₂HPO₄·7H₂O
   (5) NaCl
   (6) Tween-20
   (7) Deionized water (dH₂O)
   (8) Bleach (10% sodium hypochlorite solution)
   (9) O-phenylene diamine (OPD), Kodak lot B9A
   (10) NaC₂H₃O₂·3H₂O
   (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                Negative -
       High positive - JD 1/24/78      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 NaH$_2$PO$_4$.H$_2$O + 8.766 g NaCl, qs to 1 liter with dH$_2$O
   Solution B: 2.6807 g Na$_2$HPO$_4$.7H$_2$O + 8.766 g NaCl, qs to 1 liter with dH$_2$O

   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 is needed:

<table>
<thead>
<tr>
<th>No. of strips:</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
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<td>PBS/T</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>13</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>BSA</td>
<td>40</td>
<td>70</td>
<td>100</td>
<td>130</td>
<td>200</td>
<td>260</td>
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(6) Toxoplasma antigen (1:100):

<table>
<thead>
<tr>
<th>No. of strips:</th>
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<th>4</th>
<th>6</th>
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<td>PBS/T</td>
<td>1.5</td>
<td>3.0</td>
<td>4.0</td>
<td>5.5</td>
<td>8.0</td>
<td>9.9</td>
</tr>
</tbody>
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(7) OPD Substrate

   0.05 M NaAc buffer: 6.8 g NaC$_2$H$_3$O$_2$·3H$_2$O in 1 liter dH$_2$O
   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% H$_2$O$_2$

(8) 4 M H$_2$SO$_4$

   22.2 ml concentrated (18 M) H$_2$SO$_4$
   77.8 ml H$_2$O

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

   (3) The antigen-sensitized plates must be stored at 4°C. Expiration date is 12 months post sensitization.
(4) 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°C–25°C) before use.

(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 microtubes.

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 l).
   (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.

6. Reporting and Interpreting Results
   A. Reference ranges
      The prevalence of Toxoplasma will vary significantly in different populations.
      All EIA-IgM reactions \( \geq 2.0 \) 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) \( \geq 2.0 \), it is probably a recent infection acquired within the past 6 months.

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

8. Summary Statistics and QC Graphs
   Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The
   absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative
   control mean and the positive control mean. Because the controls are read as cutoff values, plots of these
   values are not generated for QC purposes.
9. References

