REFERENCES
1. Wilson M; Remington JS; Clavet C; Varney G; Press C; Ware D. Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to Toxoplasma gondii.

INTENDED USE
The Toxoplasma IgM ELISA kit is intended for the detection of IgM antibody to Toxoplasma in human serum or plasma. For research use only, not for use in diagnostic procedures.

SUMMARY AND EXPLANATION
Toxoplasma gondii causes toxoplasmosis, a common disease that affects 30-50 of every 100 people in North America by the time they are adults. The mean source of infection is direct contact with cat feces or from eating undercooked meats. Toxoplasmosis generally presents with mild symptoms in immunocompetent individuals; in the immunocompromised individual, however, the infection can have serious consequences. Acute toxoplasmosis in pregnant women can result in miscarriage, poor growth, early delivery or stillbirth. Treatment of an infected infant will also lessen the severity of the disease as the child grows. IgG and IgM antibodies to Toxoplasma can be detected within 2-3 weeks after exposure. IgG remains positive, but the antibody level drops over time. ELISA can detect Toxoplasma IgM antibody after one year after infection in over 50% of patients. Therefore, IgM positive results should be evaluated further with one or two follow up samples if primary infection is suspected.

PRINCIPLE OF THE TEST
Diluted serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to the wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with Toxoplasma antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>Calibrator: 1 Vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Positive Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Negative Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>
MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS
1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory Use.
3. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and
   found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed
   reagents. However, 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.
4. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting
   as well as following the exact time and temperature requirements is essential.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit
   reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of
   different lots should not be mixed.
7. 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.

REAGENT PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or
deonized water. Store at room temperature (18-26 °C).

ASSAY PROCEDURE
Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test
   samples, by adding 10 μl of the sample to 200 μl of sample diluent. Mix well.
3. Dispense 100 μl of diluted sera, calibrator and controls into the appropriate wells. For the
   reagent blank, dispense 100μl sample diluent in 1A well position. Tap the holder to remove
   air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 μl of 1X wash buffer. Blot on
   absorbance paper or paper towel.
5. Dispense 100 μl of enzyme conjugate to each well and incubate for 20 minutes at room
   temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 μl of 1X wash
   buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 μl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 μL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended
   with reference filter of 600-650 nm.

CALCULATION OF RESULTS
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to
   lot. Make sure you check the value on every kit.
2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each
   sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Sample O.D. = 1.6

LIMITATIONS OF THE TEST
1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been
   formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be
   seen after diluting serum with sample diluent. This turbidity is due to the blocking of
   serum IgG and shows no interference with test results. It can be removed by
   centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating
   the interferences cannot be ruled out entirely.
3. Lipemic or hemolyzed samples may cause erroneous results.