



Toxoplasma IgM EIA kit

(For in vitro diagnostic use only)

Enzyme-linked immunosorbent assay for the detection of IgM antibody to *Toxoplasma gondii*

INTENDED USE

The Toxoplasma IgM kit is intended for use in the detection of IgM antibodies to *Toxoplasma gondii* infection.

SUMMARY AND PRINCIPLE OF THE TEST

Toxoplasma gondii is a coccidian parasite initially isolated in 1908 from a North African rodent--the gondii. Since then, the organism has been found in many species of birds, reptiles and mammals.

Man is infected with *Toxoplasma gondii* from various suspected sources: ingestion of infected meat, especially mutton and pork, or ingestion of soil contaminated by oocyst from domestic and feral cats. Transmission by organ transplant, transfusion or activation of quiescent infections is also documented congenital Toxoplasmosis is a disease with an extraordinarily wide range of manifestations; so wide in fact, that it must be considered in the differential diagnosis of nearly all types of obscure illness occurring during infancy .

Because symptoms are sometimes nonspecific (i.e., anemia, splenomegaly, jaundice, fever, hepatomegaly, adenopathy and vomiting), congenital Toxoplasmosis is easily misdiagnosed on clinical grounds, even in sick infants who have the generalized form of the disease. Toxoplasmosis must also be considered in the differential diagnosis in any immunosuppressed patient who has clinical or laboratory evidence of damage to the central nervous system. This organism is one of the most common latent infectious agents of man throughout the world.

In acquired Toxoplasmosis, levels of IgM antibody are generally detectable very early in the infection and peak within one or two months after clinical onset. They typically remain detectable for only a few weeks, but can persist for as long as 2 years .

ATLAS TOXO IgM Capture kit utilizes ELISA based on the antibody-capture technique. Patient sera are incubated with mouse monoclonal antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. Patient anti-TOXO IgM antibodies are 'detected' and bound by an immunocomplex, Enzyme conjugate, consisting of TOXO antigen which is conjugated to horseradish peroxidase. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm and is directly proportional to the concentration of IgM antibodies to TOXO antigen present in the sample.

REAGENTS

Materials provided with the kits:

1. 8X12 well microtiter strip: 1 plate, coated with Anti-Human IgM
2. Negative Control
3. Positive Control
4. Enzyme Conjugate: HRP-conjugated-TOXO antigen
5. Wash Buffer: PBS, Tween. The buffer should be diluted with distilled water 1:20 before use.
6. Substrate Solution A: urea peroxide.
7. Substrate Solution B: TMB.
8. Stop Solution: 2N Sulfuric Acid

Materials required but not provided:

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450nm wavelength
8. Timer

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 50 µl of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50µl of serum or plasma samples into respective test wells
3. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
4. Add 1 drop (50 µl) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
5. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.

6. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
7. Add one drop (50 µl) of Substrate Solution A (HRP substrate) to each well, then add one drop (50 µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
8. Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Cut Off: 0.10 + Average OD value of Negative Control

Positive: OD value is equal to or greater than the Cut Off value

Negative: OD value is less than the Cut Off value

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE ASSAY

1. To prevent false negative and false positive IgM test results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

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