

TOXOPLASMA IgM ELISA TEST SYSTEM

INTENDED USE

The Reactiva Search, Toxoplasma IgM ELISA Test System is intended for the detection of IgM antibody to Toxoplasma in human serum or plasma.

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 main 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 patient, 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 pregnant woman may prevent or lessen the disease in her unborn child. 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 with 2-3 weeks after exposure. IgG remains positive, but the antibody level drops overtime. 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 patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to 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 AND COMPONENTS

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|---|--------|
| • Microwells coated with Toxoplasma antigen | 12x8x1 |
| • Sample Diluent: 1 bottle (ready to use) | 22ml |
| • Calibrator: 1 Vial (ready to use) | 1ml |
| • Positive Control: 1 vial (ready to use) | 1ml |
| • Negative Control: 1 vial (ready to use) | 1ml |
| • Enzyme conjugate: 1 bottle (ready to use) | 12ml |
| • TMB Substrate: 1 bottle (ready to use) | 12ml |
| • Stop Solution: 1 bottle (ready to use) | 12ml |
| • Wash concentrate 20X: 1 bottle | 25ml |

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

STORAGE CONDITIONS

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

PRECAUTIONS

1. 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.
2. 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.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. 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

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 deionized water. Store at room temperature (18- 26°C).

TEST PROCEDURE

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. Mixwell.
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

2. check the value on every kit.
3. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
4. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off
5. value.

EXAMPLE OF RESULTS AND CALCULATIONS

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 Patient sample O.D. = 1.6 Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

INTERPRETATION

The following is intended as a guide to interpretation of Toxoplasma IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9 No detectable antibody to Toxoplasma IgM by ELISA. 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.

>1.1 Detectable antibody to Toxoplasma IgM by ELISA

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. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY

178 patient sera were tested by this Toxoplasma IgM ELISA and a reference ELISA method. 26 sera were positive and 149 were negative by both methods (98% agreement). The results are summarized below:

Reactiva Search Toxoplasma IgM ELISA				
		+	-	Total
Reference ELISA Kit	+	23	2	25
	-	1	152	153
Total		24	154	178

2. PRECISION

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.89	0.14	6.35
2	16	1.16	0.048	4.14
3	16	0.21	0.012	5.71

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.76	0.150	08.52
2	10	1.22	0.120	09.83
3	10	0.18	0.021	11.67

REFERENCES

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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET099-2