

<p>CALDON BIOTECH INC. Toxoplasma IgM ELISA</p>
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Catalog No. TX024M
(96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH INC (CBI), Toxoplasma IgM ELISA is intended for the use in the evaluation of patients with suspected Toxoplasma infection.

SUMMARY AND EXPLANATION OF THE TEST

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 meansource 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 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 PROVIDED

- Microwell Strips: Toxoplasma coated wells (12 x 8 x 1 wells)
- Sample Diluent: 1 bottle (22 mL). Ready to use.
- Enzyme conjugate: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Stop solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
- Calibrator: White Cap. (1.50 mL/vial). Ready to use.
- Positive control: Red Cap. (1.50 mL/vial). Ready to use.
- Negative control: Blue Cap. (1.50 mL/vial). Ready to use.
- Wash Concentrate: 1 bottle (50 mL): 20X Concentrate.

STORAGE AND STABILITY

- 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 during storage or usage.

WARNINGS AND 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 spec imens 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. Control sera and sample diluent contain 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 of serum sample.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20-25 ° C) and gently mix.
2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

ASSAY 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. Mix well.
3. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µL 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. Discard liquid from all wells. Wash each well three times with 300 µL wash buffer.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Discard liquid from all wells. Wash each well three times with 300 µL wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. within 30 min at 450 nm using microwell reader.

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 the 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

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:

- The O.D. of the Calibrator should be greater than 0.250.
- The Ab index for Negative control should be less than 0.9.
- The Ab Index for Positive control should be greater than 1.2.

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 recommend if clinically indicated.

>1.1 Indicative of Toxoplasma infection.

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 and Specificity**

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:

Agean region, Turkey. J Egypt Soc Parasitol
1997;27(2):439-43.

	Toxoplasma IgM		
	ELISA		
	23		2
25			
	1		152
153			
	24		154
178			

**2. Precision
Intra-Assay Study**

		1.89	0.14	6.35
		1.16	0.048	4.14
		0.21	0.012	5.71

Inter-Assay Study

	10	1.76	0.150	08.52
	10	1.22	0.120	09.83
	10	0.18	0.021	11.67

REFERENCES

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