9051 Mira Mesa Blvd, #910094
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Toxoplasma IgM ELISA Kit

Name	Toxoplasma IgM ELISA Kit
INTENDED USE	The Toxoplasma IgM ELISA Kit 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 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 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	96 Tests
Microwells coated with Toxoplasma antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
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 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 AND STABILITY

Store the kit at 2-8C.
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.

WARNINGS AND PRECAUTIONS

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. 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. 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 deionized water. Store at room temperature (18-26C).
ASSAY PROCEDURE	Bring all specimens and kit reagents to room temperature (18-26 C) and gently mix. Place the desired number of coated strips into the holder. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well. Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l 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. Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l of enzyme conjugate to each well and incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 l of stop solution. 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	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. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). 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
LIMITATIONS OF THE TEST	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.

In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.

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.

Lipemic or hemolyzed samples may cause erroneous results.

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.

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

1.1 Detectable antibody to Toxoplasma IgM by ELISA

PERFORMANCE CHARACTERISTICS

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:

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

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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

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. The FDA Toxoplasmosis Ad Hoc Working Group.

Obwaller A; Hassl A; Picher O; Asp¨ock H. An enzyme-linked immunosorbent assay with whole trophozoites of Toxoplasma gondii from serum-free tissue culture for detection of specific antibodies. Parasitol Res 1995;81(5):361-4.

Loyola AM; Durighetto AF Jr; Silva DA; Mineo JR. Anti-Toxoplasma gondii immunoglobulins A and G in human saliva and serum. J Oral Pathol Med 1997; 26(4):187-91.

Doehring E; Reiter-Owona I; Bauer O; Kaisi M; Hlobil H; Quade G; Hamudu NA; Seitz HM. Toxoplasma gondii antibodies in pregnant women and their newborns in Dar es Salaam, Tanzania. Am J Trop Med Hyg 1995;52(6):546-8.

Cotty F; Descamps P; Body G; Richard-Lenoble D. Prenatal diagnosis of congenital toxoplasmosis: the role of

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Toxoplasma IgA antibodies in amniotic fluid [letter]. J Infect Dis 1995;171(5):1384-5.

Altintas N; Kuman HA; Akisu C; Aksoy U; Atambay M. Toxoplasmosis in last four years in Agean region, Turkey. J Egypt Soc Parasitol 1997;27(2):439-43.