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CALBIOTECH
A life science company

Salmonella Typhi IgM ELISA
Catalog No. ST084M (96 Tests)

INTENDED USE
The Calbiotech Inc. (CBI) Salmonella IgM ELISA Kit is intended for the detection of IgM antibody to Salmonella in human serum or plasma.

SUMMARY AND EXPLANATION
Salmonella typhi is the causative agent of typhoid fever a contagious infection of the intestines that affects the whole body. In developing countries, typhoid often occurs in epidemics. Most people in the United States get typhoid as a result of visiting another country where the food or water supply has been contaminated. Symptoms usually start 1 to 3 weeks after exposure to the bacteria. Symptoms include: high fever, headache, sore throat, vomiting, diarrhea, skin rash and weakness. The symptoms may take 2 weeks or more to go away. Typhoid is spread when a person drinks or eats food and water contaminated by human waste (stool or urine) containing *Salmonella typhi* bacteria. A person who no longer has symptoms may still transmit the bacteria as a carrier. Testing for immunoglobulin G (IgG), IgA, and IgM antilipopolysaccharide (LPS) of *Salmonella* typhi antibodies by enzyme-linked immunosorbent assay (*ELISA*) showed that the levels of all three classes of immunoglobulin anti-LPS of S. typhi were higher in typhoid patients than in healthy or febrile nontyphoidal groups. The *ELISA* assay was much more sensitive and specific than any combination of the Widal test, and hence it could be a useful tool for the serologic diagnosis of typhoidal fever with a single blood sample.

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
1.	Microwells coated with *Salmonella typhi*	12x8x1
2.	Sample Diluent: 2 bottle (ready to use)	25 ml
3.	Calibrator: 1 Vial (ready to use)	1ml
4.	Positive Control: 1 vial (ready to use)	1ml
5.	Negative Control: 1 vial (ready to use)	1ml
6.	Enzyme conjugate: 1 bottle (ready to use)	12ml
7.	TMB Substrate: 1 bottle (ready to use)	12ml
8.	Stop Solution: 1 bottle (ready to use)	12ml
9.	Wash concentrate 20X: 1 bottle	25ml

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. 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. 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.

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 (20-25°C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°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 1:101 dilution of test samples, by adding 5 µl of the sample to 0.5 mL 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 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:
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 *Salmonella typhi* 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 *Salmonella typhi* IgM by ELISA.
0.9-1.1   Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1   Detectable antibody to *Salmonella typhi* 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 has shown 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**
   89 patient sera were tested by this *Salmonella typhi* IgM ELISA and a reference ELISA method. 12 sera were positive and 72 were negative by both methods (94% agreement). The results are summarized below:

		Salmonella typhi IgM ELISA		
		+	−	Total
Reference ELISA Kit	+	12	2	14
	−	3	72	75
	Total	15	74	89

2. **Precision**

Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.75	0.079	4.51
2	16	1.22	0.068	5.57
3	16	0.28	0.024	8.57

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.68	0.123	7.32
2	10	1.06	0.087	8.20
3	10	0.35	0.037	10.57