



Salmonella IgM ELISA Kit

Catalog Number KA0966

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The Salmonella IgM ELISA Kit is intended for the detection of IgM antibody to Salmonella in human serum or plasma.

Background

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 Assay

Diluted human serum (serum diluent contains sorbent to move 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.

General Information

Materials Supplied

List of component

Component	Amount
Microwells coated with <i>Salmonella typhi</i>	96 (12x8) wells
Sample Diluent (Ready to use)	25 mL x 2
Calibrator (Ready to use)	1 mL
Positive Control (Ready to use)	1 mL
Negative Control (Ready to use)	1 mL
Enzyme conjugate (Ready to use)	12 mL
TMB Substrate (Ready to use)	12 mL
Stop Solution (Ready to use)	12 mL
20X Wash concentrate	25 mL

Storage Instruction

- ✓ 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 reagent to heat, sun, or strong light.

Materials Required but Not Supplied

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

Precautions for Use

- ✓ 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 this protocol. Precise pipetting, as well as following the exact time and temperature requirements prescribed are 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.
- ✓ 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.
- ✓ 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 has shown 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.
 - Lipemic or hemolyzed samples may cause erroneous results.

Assay Protocol

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).

Sample Preparation

- ✓ Collect blood specimens and separate the serum immediately.
- ✓ 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.

Assay Procedure

Bring all specimens and kit reagents to room temperature (18-26°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 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. 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.

Data Analysis

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 = $0.8 \times 0.5 = 0.400$

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 be greater than 1.2.

- ✓ 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 indicated.

>1.1 Detectable antibody to *Salmonella typhi* IgM by ELISA.

Performance Characteristics

✓ Sensitivity and Specificity

89 sera sample 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

✓ 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

Resource

Reference

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5. Vitale G; Librizzi R; Mocciaro C; Friscia I; Blandino E; Usticano V; Mansueto S; Di Fiore M; Reina G; Gambino G. An ELISA method in the diagnosis of typhoid fever. *J Clin Lab Immunol* 1990; 31(4):195-9.

Plate Layout

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