Typhoid IgG/IgM Rapid Test-Cassette (Serum / Plasma)

INTENDED USE

The Typhoid IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of anti-*Salmonella typhi* (*S. typhi*) IgG and IgM in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with *S. typhi.* Any reactive specimen with the Typhoid IgG/IgM Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

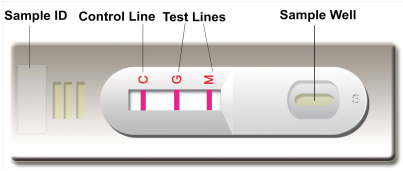
Typhoid fever is caused by *S. typhi*, a Gram-negative bacterium. World-wide an estimated 17 million cases and 600,000 associated deaths occur annually[1]. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S. typhi*[2]. Evidence of *H. pylori* infection also presents an increase risk of acquiring typhoid fever. 1-5% of patients become chronic carrier harboring *S. typhi* in the gallbladder.

The clinical diagnosis of typhoid fever depends on the isolation of *S. typhi* from blood, bone marrow or a specific anatomic lesion. In the facilities that can not afford to perform this complicated and time-consuming procedure, Filix-Widal test is used to facilitate the diagnosis. However, many limitations lead to difficulties in the interpretation of the Widal test[3,4].

In contrast, the Typhoid IgG/IgM Rapid Test is a simple and rapid laboratory test. The test simultaneously detects and differentiates the IgG and the IgM antibodies to *S. typhi* specific antigen[5] thus to aid in the determination of current or previous exposure to the *S. typhi*.

TEST PRINCIPLE

The Typhoid IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *S. typhoid* H antigen and O antigen conjugated with colloid gold (Typhoid conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (M and G bands) and a control band (C band). The M band is pre-coated with monoclonal anti-human IgM for the detection of IgM anti-*S. typhi*, G band is pre-coated with reagents for the detection of IgG anti-*S. typhi* , and the C band is pre-coated with goat anti rabbit IgG.



When an adequate volume of test specimen is dispensed into the sample well of the cassette, the test specimen migrates by capillary action across the test cassette. Anti-*S. typhi* IgM if present in the patient specimen will bind to the Typhoid conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody, forming a burgundy colored M band, indicating a *S. typhi* IgM positive test result.

Anti-*S. typhi* IgG if present in the patient specimen will bind to the Typhoid conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored G band, indicating a *S. typhi* IgG positive test result.

Absence of any test bands (M and G) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 25 or 30 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One plastic dropper.
   c. One desiccant.
2. Sample Diluent (1 bottle, 5 mL)
3. One package insert (instruction for use).

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control (1 vial, red cap, 1 mL, Cat # R0160-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R0160-N)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. The positive and negative controls should be kept at 2°C-8°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately.

Store specimens at 2°C-8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 μL) of specimen into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 μL) of Sample Diluent immediately.

1 drop of specimen **1 drop of sample diluent** **15 minutes**

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C band. The C line develops after adding specimen and sample diluent. Otherwise, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performing of the assay, in particularly, under the following circumstances:
   a. New operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit fall outside of 2°C -30°C.

e. The temperature of the test area falls outside of 15°C -30°C.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE OR NON-REACTIVE RESULT**: If only the C band is present, the absence of any burgundy color in the both test bands (M and G) indicates that no anti-*S. typhi* antibody is detected in the specimen. The result is negative or non-reactive.

2. **POSITIVE OR REACTIVE RESULT**:

2.1 In addition to the presence of C band, if only M band is developed, the test indicates for the presence of anti- *S. typhi* IgM in the specimen. The result is IgM positive or reactive.

2.2 In addition to the presence of C band, if only G band is developed, the test indicates for the presence of anti- *S. typhi* IgG in the specimen. The result is IgG positive or reactive.

2.3 In addition to the presence of C band, both M and G bands are developed, the test indicates for the presence of anti-*S. typhi* IgG and IgM in the specimen. The result is both IgG and IgM positive or reactive.

Samples with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of any burgundy color in the test bands as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance For IgM Test**

A total of 334 samples from susceptible subjects were tested by the Typhoid IgG/IgM$^{2.0}$ Rapid Test and by a commercial *S. typhi* IgM EIA. Comparison for all subjects is showed in the following table.

	Typhoid IgG/IgM$^{2.0}$ Rapid Test		
IgM EIA	**Positive**	**Negative**	**Total**
Positive	31	3	34
Negative	2	298	300
Total	33	302	334

Relative Sensitivity: 91% , Relative Specificity: 99.3%, Overall Agreement: 98.5%

2. **Clinical Performance For IgG Test**

A total of 314 samples from susceptible subjects were tested by the Typhoid IgG/IgM$^{2.0}$ Rapid Test and by a commercial *S. typhi* IgG EIA kit. Comparison for all subjects is showed in the following table.

	Typhoid IgG/IgM$^{2.0}$ Rapid Test		
IgG EIA	**Positive**	**Negative**	**Total**
Positive	13	1	14
Negative	2	298	300
Total	15	299	314

Relative Sensitivity: 92.9% , Relative Specificity: 99.3%, Overall Agreement: 99.0%

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to *S. typhi* in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Typhoid IgG/IgM$^{2.0}$ Rapid Test is limited to the qualitative detection of antibodies to *S. typhi* in human serum or plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. The Typhoid IgG/IgM$^{2.0}$ Rapid Test also detects para-typhi antibodies.
4. A negative result for an individual subject indicates absence of detectable anti-*S. typhi* antibodies. However, a negative test result does not preclude the possibility of exposure to *S. typhi*.
5. A negative result can occur if the quantity of anti-*S. typhi* antibodies present in the specimen is below the detection limit of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
6. If the symptom persists, while the result from Typhoid IgG/IgM$^{2.0}$ Rapid Test is negative or non-reactive result, it is recommended to re-sample the patient few days late or test with an alternative test method, such as bacterial culture method.
7. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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