Salmonella typhi/paratyphi A, B & C Test Device

For detection of Salmonella typhi and paratyphi A, B & C antigens in stool/serum/plasma.

IVD *In-vitro diagnostic use only* *Not reuse*

Introduction

Typhoid fever is a life threatening illness caused by the bacterium Salmonella typhus, and was observed by Eberth (1880) in the mesenteric nodes and spleen of fatal cases of typhoid fever. It is common in developing countries where it affects about 12.5 million persons annually. The infection is acquired typically by ingestion. On reaching the gut, the bacilli attach themselves to the epithelial cells of the intestinal villi and penetrate the lamina and submucosa. They are then phagocytosed there by polymorphs and mesenteric lymph nodes, where they multiply and, via the thoracic duct, enter the blood stream. A transient bacteremia follows, during which the bacilli are seeded in the liver, gall bladder, spleen, bone marrow, lymph nodes, and kidneys, where further multiplication takes place. Towards the end of the incubation period, there occurs a massive bacteremia from these sites, heralding the onset of the clinical symptoms.

Serovar *paratyphi* A is the second most prevalent cause of Typhoid. *Paratyphi* A and *typhi* cause a similar illness, with relapsing fever. The diagnosis of typhoid and Paratyphoid consists of isolation of the bacilli and the demonstration of antibodies. The isolation of the bacilli is very time consuming and antibody detection is not very specific.

Our test employs a combination of monoclonal antibody/colloidal gold dye conjugate and a polyclonal antibody immobilized on the solid phase. This will selectively identify the S. *typhi* and *paratyphi* A antigens associated Salmonella *typhi* (typhoid) and salmonella *paratyphi* (paratyphoid) infections with a high degree of sensitivity and specificity.

Intended Use

The Salmonella typhi/paratyphi A, B & C Test Device is a rapid chromatographic immunoassay for the qualitative detection of Salmonella *typhi and paratyphi* A antigens in stool/serum/plasma

Materials Included and Active Ingredients

1) Test kit contains the following items to perform the assay.
   - Test device foil pouched with a desiccant
   - Assay Buffer
   - Instruction for use

Kit Precautions and Storage Instructions

1) For best results, adhere to instructions provided
2) All specimens should be handled as potentially infectious
3) The test device should be stored at room temperature
4) The test device is sensitive to humidity as well as heat
5) Do not use beyond expiration date
6) Do not use test kit if pouch is damaged or seal is broken
7) Use test device immediately after removing from the pouch
8) The components (test device and assay diluents) in this kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

Warnings

1) For in vitro diagnostic use only. DO NOT RE-USE test device
2) The instructions must be followed to obtain accurate results. Anyone performing an assay with this product must be trained in its use and laboratory procedures.
3) Do not eat or smoke while handling specimens
4) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
5) Avoid splashing or aerosol formation
6) Clean up spills thoroughly using an appropriate disinfectant
7) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
8) Do not mix with other specimens.

Specimen Collection, Storage and Precautions

1) Stool should be collected in the specimen collection container.
2) Separate the Serum or Plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
3) Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C.
4) Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
5) If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

Test Procedure

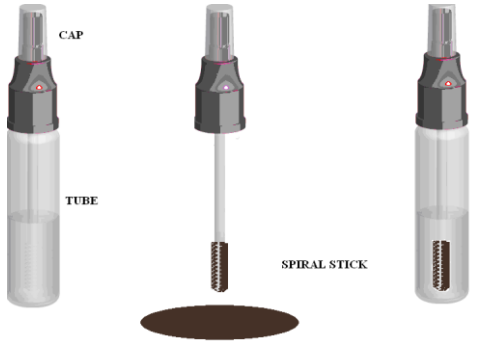
Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.

For Stool Samples Only:

SAMPLE COLLECTION METHOD

(*Please refer to illustration*)

1. Loosen cap of extraction tube and remove cap with the spiral stick.
2. Introduce the spiral stick into the stool sample six times at six different sites of the sample. Try to avoid getting clumps of sample into the spiral stick. Collect about ½ gram of stool.
3. Return the spiral stick into the extraction tube.
4. Tighten cap and shake the tube to disperse the sample evenly into the buffer.
5. Remove the test cassette from the sealed pouch.
6. Hold the extraction tube upright with the tip pointed away from the person performing the test and snap off the tip.
7. Hold the bottle in a vertical position over the sample well of the test device, deliver 3 drops (150 µL) of diluted stool sample to the sample into the well.
8. If the flow of the buffer along the test cassette appears to have stopped, lightly tap the test cassette on a hard flat surface. If flow does not continue then dispense one drop at a time until flow continues.0and 20 minutes. DO NOT INTERPRET RESULTS AFTER 30 MINUTES.



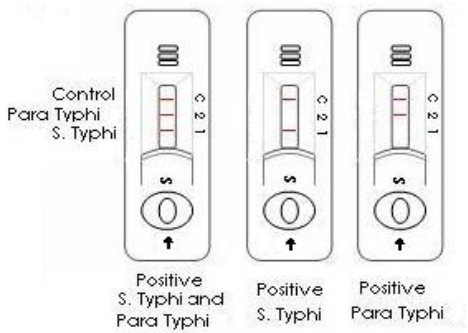
Introduce spiral stick into fecal matter at 6 different sites on the specimen.

For Serum or plasma Samples:

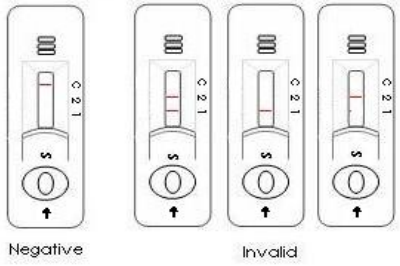
1. Add 100 µLs of serum/plasma into the sample well.

2. The result should be read between 10 to 20 minutes but not more than 30 minutes.

Interpretation of Test Results (Refer to Figure)



POSITIVE: S. *typhi/para typhi*: Three distinct red lines appear. One line should be in the control region (C) and the other two lines should be in both test regions (1&2).
S. *typhi*: Two distinct red lines appear. One line should be in the control region (C) and one line in test region 1.
paratyphi: Two distinct red lines appear. One line should be in the control region (C) and one line in test region 2.
NOTE: The intensity of the red color in the test line region (T) will vary depending on the concentration of *S. typhi* and/or *paratyphi* A antigen(s) present in the specimen. Therefore, any shade of red in the test region (T) should be considered positive.



NEGATIVE: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

NOTE*: A low S. typhi/paratyphi A, B & C concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 30 minutes.*

Limitations of the Test

1) The Salmonella *typhi/paratyphi* Test Device is for *in vitro* diagnostic use only. This test should be used for the detection of S. *typhi/paratyphi A* antigen in specimen.
2) The Salmonella *typhi/paratyphi* A, B & C Test Device will only indicate the presence of *S. typhi/paratyphi* A antigen in the specimen and should not be used as the sole criteria for the diagnosis of Typhoid and Paratyphoid infection.
3) As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4) If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Typhoid/ Paratyphoid infection.

Suggested Readings

1) Ashish P. Maskey et al. *Salmonella enterica Serovar Paratyphi A and S. enterica Serovar Typhi Cause Indistinguashable Clinical Syndromes in Kathmandu, Nepal*. CID 2006:42 (1 May).
2) 2. Fangtham, Dr. Monthida; Wilde, Dr. Henry; *Emergence of Salmonella paratyphi A as a Major Cause of Enteric Fever: Need for Early Detection, Preventative Measures, and Effective Vaccines.* Journal of Travel Medicine Vol15, Issue5, 2008 344-350.
3) 3. Wu, Weiyuan et al. *Genetic Diversity of Salmonella enteric Serovar Typhi and Paratyphi in Shenzhen, China from 2002 through 2007.* BMC Microbiology 2010, 10:32
4) 4. Baker, Stephen; Favorov, Michael,' Dougan, Gordon. *Searching for the elusive typhoid diagnostic.* BMC Microbiology 2010, 10:45

REF RT-TA1112-C-1

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