OnSite® S. Typhi/Paratyphi Ag Rapid Test

REF R0162C

Instructions for Use

INTENDED USE

The *OnSite* S. Typhi/Paratyphi Ag Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*) antigens in human fecal specimen, serum, plasma, whole blood or blood culture specimen. It is intended to be used by healthcare professionals to aid in the diagnosis of infection with *S. typhi* and/or *S. paratyphi*.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

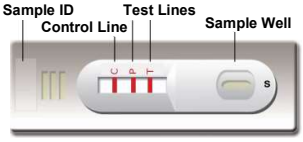
Typhoid fever and paratyphoid fever are bacterial infections caused by *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*) A, B, and C, which is transmitted through the ingestion of tainted food and water[1]. Worldwide an estimated 21 million cases and 222,000 associated deaths occur annually[2]. The majority of infections are cause by *S. typhi* with infections by *S. paratyphi* A, B, and C being more rare[2].

After the *S. typhi* or *S. paratyphi* bacteria are ingested, they enter the small intestine via the microfold cells through which the bacteria migrate to the mesenteric lymph nodes and multiply. Clinical onset of the disease presents with symptoms including fever, nausea, constipation and diarrhea. The incubation period is usually 8 to 14 days, but periods ranging from 3 days to more than 60 days have been reported. The bacteria are removed from the blood via the gall bladder to the small intestine.

The clinical diagnosis of infections depends on isolation of *S. typhi* and *S. paratyphi* from blood, bone marrow or a specific anatomic lesion. In facilities that cannot afford to perform this complicated and time-consuming procedure, tests for antibody detection can be used including the Widal test[3,4] or lateral flow rapid tests[5].

The *OnSite* S. Typhi/Paratyphi Ag Rapid Test detects the specific antigens from either *S. typhi* or *S. paratyphi* presenting in the fecal specimen, serum, plasma, whole blood or blood culture from the infected patient within 15 minutes. The test can be performed by minimally trained personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* S. Typhi/Paratyphi Ag Rapid Test is a sandwich lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing monoclonal anti-*S. typhi/paratyphi* conjugated with colloidal gold (anti-*S. typhi/paratyphi* conjugates) and 2) a nitrocellulose membrane strip containing two test lines (P and T line) and a control line (C line). The P line is pre-coated with monoclonal anti-*S. paratyphi,* The T line is pre-coated with monoclonal anti-*S. typhi*, and the C line is pre-coated with a control line antibody.

When an adequate volume of specimen or fecal sample extraction buffer is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. The *S. typhi* antigen, if present in the specimen, will bind to the anti-*S. typhi* conjugate. The immunocomplex is then captured on the membrane by the pre-coated antibody forming a colored T line, indicating a Typhi Ag positive test result.

The *S. paratyphi* antigen, if present in the specimen, will bind to the anti-*S. paratyphi* conjugate. The immunocomplex is then captured on the membrane by the pre-coated antibody forming a colored P line, indicating a Paratyphi Ag positive test result.

Absence of test lines (P and T line) suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies, regardless of the color development on the T or P line. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Stool collection devices, each containing 1 mL Fecal Sample Extraction Buffer (REF SB-R0162)
3. Sample diluent (REF SB-R0162-2)
4. Plastic droppers
5. Patient ID stickers
6. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container to hold fecal specimen
3. Lancing device for whole blood test
4. Blood culture tube and culture media

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use any kit components beyond their stated expiration date.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Bring all reagents to room temperature (15-30°C) before use.
6. ***Do not scoop fecal specimen as this may lead to excess fecal specimen that tends to clog the sample pad and interfere with sample migration.***
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 bio-safety.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Avoid extraction buffer contact with skin or eyes. Do not ingest.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. The test results should be read 15-20 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unopened test devices at 2-30°C. If stored at 2-8°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 to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Preparation of fecal extract from fecal specimen

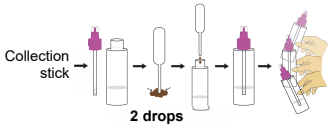
Step 1: Collect a random stool specimen in a clean, dry receptacle.
Step 2: Label the stool collection device with the specimen's ID number (patient ID sticker).

Solid fecal specimen

Step 3: Open the stool collection device by unscrewing the top and use the collection stick to randomly pierce in 2-5 different sites, twisting the collection stick into the fecal specimens to help collection if necessary. ***Do not scoop fecal specimen as this may lead to an invalid test result.***
Step 4: Ensure that all inner grooves of the collection stick are filled with fecal specimen. However, excess fecal specimen on the outside of grooves should be scraped off. ***Incorrect sampling may lead to an erroneous test result.***

Watery fecal specimen

Step 3: Open the stool collection device by unscrewing the top.
Step 4: Fill the plastic dropper with the watery fecal specimen; dispense 2 drops (70-85 µL) into the stool collection device.



The specimen is now ready for testing, transportation or storage.

Step 5: Replace the collection stick and tighten securely to close the stool collection device.
Step 6: Shake the stool collection device vigorously.

Note: The extracted specimens may be stored at 2-8°C or at room temperature for a few days. For longer storage, the extracted specimen may be frozen at -20°C. Avoid multiple freeze-thaw cycles.

Preparation of serum, plasma, and whole blood specimen

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for 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 with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

Preparation of blood culture specimen

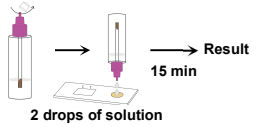
Blood specimen is cultured in suitable culture media at desirable time with the procedure established in individual laboratory.

TEST PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.
Step 2: When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.
Step 3: Be sure to label the device with the specimen ID number.
Step 4:

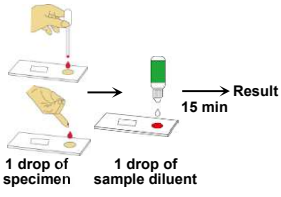
For solid/watery fecal specimen

4.1 Shake the stool collection device vigorously to ensure a homogenous liquid suspension.
4.2 Hold the stool collection device vertically. Twist off the cap. Dispense 2 drops (70-90 µL) of the solution into the center of the sample well of the cassette, making sure that there are no air bubbles. Do not overload the solution.



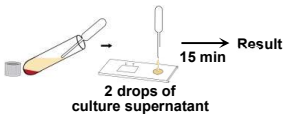
For serum/plasma/whole blood specimen

4.1 Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of serum/plasma or 1 drop of whole blood (about 40-50 µL) into the sample well making sure that there are no air bubbles.
4.2 Immediately add 1 drop (about 35-50 µL) of sample diluent to the sample well with bottle positioned vertically.



For blood culture specimen

4.1 At the end of culture period, the red blood cells will settle to the bottom of tube. Fill the plastic dropper with the supernatant, do not disturb the blood cells.
4.2 Hold the dropper vertically, dispense 2 drops (80-90 µL) of culture supernatant into the center of the sample well, making sure that there are no air bubbles.



Step 5: Set up the timer.
Step 6: Results can be read at 15 minutes. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated. Discard used device after interpreting the result following local requirements governing the disposal of device.***

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen extract. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. External Control: Good Laboratory Practice recommends using external positive and negative controls to assure the proper performance of the assay, particularly under the following circumstances:

a. A new operator uses the kit, prior to performing testing of specimens.
b. A new lot of test kits is used.
c. A new shipment of test kits is used.
d. The temperature during storage falls outside of 2-30°C.
e. The temperature of the test area falls outside of 15-30°C.
f. To verify a higher than expected frequency of positive or negative results.
g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT: If only the C line develops, the test indicates that no detectable *S. typhi/paratyphi* antigens are present in the specimen. The result is negative or non-reactive.

2. POSITIVE RESULT:

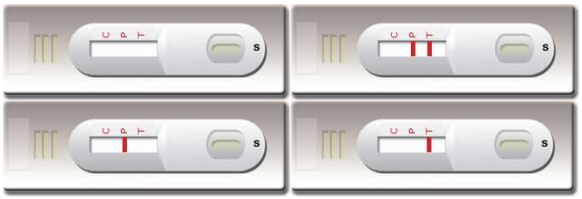
2.1 If both C and P lines develop, the test indicates the presence of detectable *S. paratyphi* antigen in the specimen. The result is S. Paratyphi Ag positive or reactive.

2.2 If both C and T lines develop, the test indicates the presence of detectable *S. typhi* antigen in the specimen. The result is S. Typhi Ag positive or reactive.

2.3 If C, P and T lines all develop, the test indicates the presence of both detectable *S. typhi* and *S. paratyphi* antigen in the specimen. The result is both S. Typhi and S. Paratyphi Ag positive or reactive.

Specimens with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

3. INVALID: If no C line develops, the assay is invalid regardless of any color development on the T or P line as indicated below. Repeat the assay with a new test device. **Excess fecal specimen can lead to invalid test results; if this is the cause, *re-sample and re-test (see instructions for collection of specimen).***



PERFORMANCE CHARACTERISTICS

1. Limit of Detection

Fecal specimen extracts, serum, plasma, whole blood and blood cultures collected from 20 healthy individuals were spiked with *S. typhi, S. paratyphi A and S. paratyphi B*, respectively, and tested with the *OnSite* S. Typhi/Paratyphi Ag Rapid Test. The detection limit is defined as the minimum titer of bacteria (organism/mL) with ≥95% positive detection rate. The results were showed in the table below.

	S. typhi	*S. paratyphi A*	*S. paratyphi B*
Fecal specimen	1x10^5	1x10^7	1x10^6
Serum Specimen	1x10^6	1x10^8	1x10^7
Plasma Specimen	1x10^6	1x10^7	1x10^7
Whole blood	1x10^6	1x10^7	1x10^7
Blood culture	1x10^5	1x10^7	1x10^6

2. Cross-Reactivity

2.1 Fecal Specimen

The organisms listed below were tested for cross-reactivity with the *OnSite* S. Typhi/Paratyphi Ag Rapid Test. No cross-reactivity was observed with organisms at 1 x 10^8 organism/mL.

Acinetobacter calcoaceticus
Adenovirus
Enterococcus faecalis
Escherichia coli
Gardnerella vaginalis
Geotrichum candidum
Helicobacter pylori
Klebsiella pneumoniae
Moraxella catarrhalis
Neisseria gonorrheae
Neisseria meningitides
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Rotavirus

2.2 Serum/Plasma Specimen

No false positive results were observed from the following non-typhoid febril diseases or specific conditions.

Dengue	Leishmania	Malaria	Toxo
TB	ANA	HAMA	RF (≤ 4,200 IU/mL)

3. Interference

Common substances (such as antibiotics) may affect the performance of *OnSite* S. Typhi/Paratyphi Ag Rapid Test. This was studied by spike these substances into negative, weak positive and medium positive specimens, respectively. The results demonstrate that at the concentration tested, the substances studied do not affect the performance of *OnSite* S. Typhi/Paratyphi Ag Rapid Test.

List of potentially interfering substances and concentrations tested.

1. Albumin	60 g/L	7. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	8. Heparin	3000 U/L
3. Creatinine	442 µmol/L	9. Metacortandracine	2 mg/mL
4. Dexamethasone	0.5 mg/mL	10. Norfloxacin	2 mg/mL
5. EDTA	1.1 µmol/L	11. Salicylic acid	4.34 mmol/L
6. Glucose	55 mmol/L	12. Sodium citrate	3.8%

4. Clinical Performance

A total of 96 characterized fecal specimens were tested by the *OnSite* S. Typhi/Paratyphi Ag Rapid Test. Test performance summarized below:
Sensitivity: 92.3% (95% CI: 74.9-99.1%)
Specificity: 97.1% (95% CI: 90.0-99.7%)
Overall agreement: 95.8% (95% CI: 89.7-98.9%)

5. Hook Effect

No hook effect was detected at concentration up to 10^9 organism/mL.

LIMITATIONS OF THE TEST

1. The Test Procedure and the Interpretation of Assay Result must be followed closely when testing the presence of *S. typhi* and *S. paratyphi* antigens in human fecal specimen, serum, plasma, whole blood, or blood culture. Failure to follow the procedure, particularly the Specimen Collection and Handling procedure, may lead to inaccurate results.
2. The *OnSite* S. Typhi/Paratyphi Ag Rapid Test is limited to the qualitative detection of *S. typhi* and *S. paratyphi* antigens in human fecal specimen, serum, plasma, whole blood and blood culture. The intensity of the test line does not have linear correlation with antigen concentration in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable *S. typhi* and *S. paratyphi* antigens. However, a negative or non-reactive test result does not preclude the possibility of infection with *S. typhi* or *S. paratyphi*.
4. A negative or non-reactive result can occur if the quantity of the *S. typhi* or *S. paratyphi* antigens present in the specimen is below the detection limits of the assay, or the antigens that are detected are not present in the test specimens, such as from patient under antibiotic treatment prior to taking of specimens.
5. If initial test result with serum, plasma or whole blood on *OnSite* S. Typhi/Paratyphi Ag rapid test is negative, while symptoms are highly suspicious, detection with *OnSite* S. Typhi/Paratyphi Ag rapid test on fecal specimens or culture is recommended.
6. *OnSite* S. Typhi/Paratyphi Ag rapid test might be able to detect *S typhi* or *S. paratyphi* antigen in the blood cultured less than 12 hours. Individual laboratory can adjust own culture procedure.
7. Infection may progress rapidly. If the symptom persists, while the result from the *OnSite* S. Typhi/Paratyphi Ag Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method, such as culture.
8. Ability to detection of *S. paratyphi* C by *OnSite* S. Typhi/Paratyphi Ag Rapid Test is not confirmed.
9. Unusually high titers of heterophile antibodies or rheumatoid factor (>4,200 IU/mL) may affect expected results.
10. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Ivanoff BN, Levine MM, Lambert PH. Vaccination against typhoid fever: present status. Bulletin of the World Health Organization 1994; 72: 957-71.
2. Immunization, Vaccines and Biologicals. World Health Organization (WHO). http://www.who.int/immunization/diseases/typhoid/en/
3. Clegg A, Passey M, Omena MK, et al. Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. Acta Tropica 1994;57:255-63.
4. Pang T. False positive Widal test in nontyphoid Salmonella infection. Southeast Asian Journal of Tropical Medicine and Public Health 1989; 20: 163-4.
5. Bhutta Z A. Current concepts in the diagnosis and treatment of typhoid fever. BMJ. 2006;333(7558): 78–82.

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	Tests per kit
2°C 30°C	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

PI-R0162C Rev. A2.0
Date released: 2020-11-12
English version

For Export Only, Not for Re-sale in the USA.