Dia Sure

Salmonella Typhi/ Paratyphi Antigen Rapid Test (Feces)

INTENDED USE

The *Salmonella Typhi/ Paratyphi* Antigen Rapid Test (Feces) is a rapid visual immunoassay for the qualitative presumptive detection and differentiation of *S. typhi* and/or *S. paratyphi* A antigens in human fecal specimens. This kit is intended to be used as an aid in the detection and differentiation of *S. typhi* and *S. paratyphi A* associated with typhoid fever or paratyphoid fever with a high degree of sensitivity and specificity.

INTRODUCTION

Salmonella enterica subsp *enterica* contains a large number of serovars accounting for more than 99.5% of isolated Salmonella strains. Among these serovars, *S. enterica* subsp. *enterica* serovar Typhi and *S. enterica* subsp. *enterica* serovar Paratyphi, often called as *S. typhi* and *S. paratyphi* respectively for short, are of important clinical significance, since these two serovars are associated with typhoid fever and paratyphoid fever. Typhoid fever, also named typhoid, is a bacterial infection due to *S. typhi*. Symptoms may vary from mild to severe and usually begin six to thirty days after exposure. For patients with typhoid, *S. typhi*, growing in the intestines and blood, can be transmitted by oral-fecal route[1]. Paratyphoid, another type of enteric fever, is caused by *S. paratyphi* A, B or C. Paratyphoid resembles typhoid in signs and symptoms.

While antibiotics have markedly reduced the frequency of typhoid in developed nations, it remains endemic in developing countries[2]. In contrast to nontyphoidal salmonellae, *S. typhi* and *S. paratyphi* enter the host's system primarily through the distal ileum. With specialized fimbriae that adhere to the epithelium over clusters of lymphoid tissue in the ileum, the main relay point for macrophages traveling from the gut into the lymphatic system. The bacteria then induce their host macrophages to attract more macrophages[3]. The ability to resist intracellular killing and to multiply within these cells is a measure of the bacterial virulence. They enter the mesenteric lymph nodes where they multiply, and via the thoracic duct, enter the blood stream. A transient bacteremia follows, heralding the onset of the clinical symptoms.

Chronic carriers are responsible for much of the transmission of the organism. While asymptomatic, they may continue to shed bacteria in their stool for decades. The bacteria excreted by a single carrier may have multiple genotypes, making it difficult to trace an outbreak to its origin[4]. Therefore, diagnosis of such pathogens will not only provide an aid in treatment therapy, but also reduce the transmission risk from symptomatic patients and chronic carriers to other population.

The diagnosis of typhoid 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. Other serological tests for antibody detection including the Widal reaction also show poor sensitivity and specificity. The *S. Typhi + S. Paratyphi* Rapid Test takes only 10-20 minutes and requires only a small quantity of human feces to perform. It is the easiest and most specific method to detect and differentiate *S. typhi* and *S. paratyphi A* infection.

PRINCIPLE

The *Salmonella Typhi/ Paratyphi* Antigen Rapid Test (Feces) has been designed to detect *S. typhi* and/or *S. paratyphi* through visual interpretation of color development in the internal strip. Anti-*S. typhi* and anti-*S. paratyphi* monoclonal antibodies are immobilized on the respective test regions of the nitrocellulose (NC) membrane. A fecal sample is added to the sample diluent buffer which is optimized to extract the *S. typhi* and/or *S. paratyphi* antigens from specimen. During testing, the extracted antigens, if present, will bind to anti-*S. typhi* and/or *S. paratyphi* antibodies conjugated to colored particles on the sample pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the NC membrane, the complex will be captured by anti-*S. typhi* and/or *S. paratyphi* antibodies at the detection zone. Presence of a colored band indicates a positive result, while its absence indicates a negative result. Excess colored particles are captured at the internal control zone as well as indicating that proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

Materials Provided

- Individually packed test devices
- Droppers
- Sample dilution tube with buffer
- Package insert

Materials Required but Not provided

- Specimen collection container
- Centrifuge
- Clock, timer or stopwatch
- Disposable latex gloves

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use the test after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- Used testing materials should be discarded according to local regulations.

STORAGE AND STABILITY

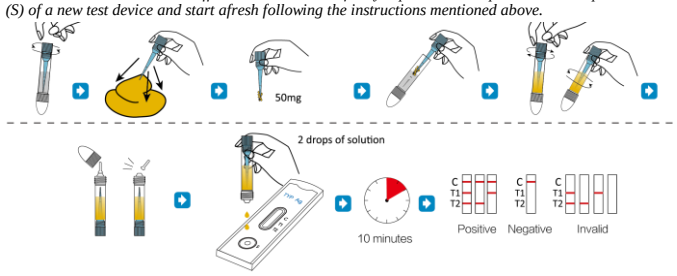
- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **DO NOT FREEZE.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

TEST PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

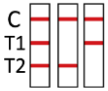
1. Specimen collection and pre-treatment:
   1) Use clean, dry specimen containers for specimen collection. Best results will be obtained if the assay is performed within one hour after collection.
   Note: If not tested within one hour, specimens collected in the specimen container may be stored for 1~2 days at 2~8°C. For long-term storage, specimens should be kept below -20°C.
   2) **For solid specimens:** Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 3 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
   For liquid specimens: Hold the dropper vertically, aspirate fecal specimens, and then transfer 3 drops (approximately 100uL) of the liquid specimen into the sample diluent tube.
   3) Place the applicator back into the tube and screw the cap tightly. Be careful not to break the tip of the dilution tube.
   4) Shake the specimen collection tube to mix the specimen and the diluent buffer thoroughly.
2. Testing
   1) Remove the test from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. To obtain a best result, the assay should be performed within one hour.
   2) Using a piece of tissue paper, break the tip of the dilution tube. Hold the tube vertically and dispense 2 drops of solution into the specimen well (S) of the test device.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
   As the test begins to work, you will see color move across the membrane.
3. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

Note: *If the specimen does not migrate (presence of particles), centrifuge the extracted specimens contained in the extraction buffer vial. Collect 100 µL of supernatant, dispense into the specimen well (S) of a new test device and start afresh following the instructions mentioned above.*



RESULT INTERPRETATION

POSITIVE RESULT:



***S. typhi* + *S. paratyphi* A Positive:** One red band appears in the control region (C), and two other red bands appear in both T1 region and T2 region. The shade of color may vary from pink to purple, but it indicates a positive result even with a faint line.

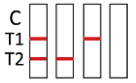
***S. typhi* Positive:** One red band appears in the control region (C), and another red band in the T1 region. The shade of color may vary from pink to purple, but it indicates a positive result even with a faint line.

***S. paratyphi A* Positive:** One red band appears in the control region (C), and another red band in the T2 region. The shade of color may vary from pink to purple, but it indicates a positive result even with a faint line.

NEGATIVE RESULT:

Negative: Only one red band appears in the control region (C), and no band appears either in the T1 region or T2 region.

INVALID RESULT:



Invalid: No red band appears in the control region (C), whether a test band(s) is present or not. Repeat invalid tests with a new sample, new test device and reagent. Insufficient sample volume, inaccurate operating procedure or expired tests may yield an invalid result. Contact your local distributor if the problem continues.

NOTE:

1. The intensity of color in the test regions (T1 and T2) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The *Salmonella Typhi/ Paratyphi* Antigen Rapid Test (Feces) is for professional *in vitro* diagnostic use, and should be used for the qualitative detection and differentiation of *S. typhi* and *S. paratyphi* antigens only.
2. Following certain antibiotic treatments, the concentration of *S. typhi* or *S. paratyphi* antigens may decrease to the concentration below the minimum detection level of the test. Therefore, diagnosis should be made with caution during antibiotic treatment.
3. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
4. A high dose "hook effect" may occur where the color intensity of test band decreases as the concentration of antigen increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the test band.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

214 patient fecal samples were collected and tested on the *Salmonella Typhi/ Paratyphi* Antigen Rapid Test and a commercial *Salmonella typhi and paratyphi* antigen rapid test. Comparison for all subjects is shown in the following table:

	Salmonella Typhi/ Paratyphi Antigen Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	89	1	90
Negative	1	123	124
Total	90	124	214

Relative Sensitivity: 98.9%
Relative Specificity: 99.2%
Overall Agreement: 99.1%

Specificity:

Cross reactivity with following organisms has been studied at 1.0 X 10^9 organisms/mL. The following organisms were found negative when tested with the *Salmonella Typhi/ Paratyphi* Antigen Rapid Test (Feces).

Shigella spp	*Campylobacter* spp	*Escherichia coli*
Helicobacter pylori	*Enterococcus faecalis*	*Enterococcus faecium*
Norovirus	*Proteus mirabilis*	*Clostridium difficile*
Staphylococcus aureus	*Rotavirus*	*Adenovirus*
Pseudomonas aeruginosa	*Neisseria meningitidis*	*Neisseria gonorrhea*
Group C Streptococcus	*Gardnerella vaginalis*	*Group B Streptococcus*
Klebsiella pneumoniae	*Candida albicans*	

LITERATURE REFERENCES

1. "Typhoid vaccines: WHO position paper.". Wkly Epidemiol Rec. 83 (6): 49–59. Feb 8, 2008.
2. Christie AB. Infectious Diseases: Epidemiology and Clinical Practice. 4th ed. Edinburgh, Scotland: Churchill Livingstone; 1987.
3. Raffatellu M, Chessa D, Wilson RP, Tükel C, Akçelik M, Bäumler AJ. Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. *Infect Immun*. 2006 Jan. 74(1):19-27.
4. Chiou CS, Wei HL, Mu JJ, Liao YS, Liang SY, Liao CH, et al. Salmonella enterica serovar Typhi variants in long-term carriers. *J Clin Microbiol*. 2013 Feb. 51(2):669-72.

GLOSSARY OF SYMBOLS

ρ	Catalog number	0	Temperature limitation
ι	Consult instructions for use	Λ	Batch code
I	In vitro diagnostic medical device	ε	Use by
μ	Manufacturer	T	Contains sufficient for <n> tests
σ	Do not reuse	A	Authorized representative in the European Community
Y	CE marking according to IVD Medical Devices Directive 98/79/EC		

Dia Sure

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