Rapid Response®

***Salmonella Typhi/Paratyphi* Test Cassette**
(Feces)
REF SAL-9C40
SAL-9C25
SAL-9C

Product Insert

For professional laboratory *in-vitro* diagnostic use only.

Intended Use

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

Introduction

Salmonella enterica, subspecies *enterica,* contains a large number of serovars accounting for more than 99.5% of isolated Salmonella strains. Among these serovars, *S. enterica,* subspecies *enterica* serovar Typhi, and *S. enterica,* subspecies *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. *S. typhi* and *S. paratyphi* have specialized fimbriae that adhere to the ileal epithelium over clusters of lymphoid tissue – a 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 for *S. typhi* and *S. paratyphi* bacteria to resist intracellular killing and to multiply within these cells acts as a measure of the bacterial virulence. They then 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 populations.

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 Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette takes only 10 minutes and requires only a small quantity of human feces to perform. It is the easiest and most specific method for detecting *S. typhi* and *S. paratyphi* infection.

Principle

The Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette has been designed to detect *S. typhi* and/or *S. paratyphi* through visual interpretation of color development in the internal strip. Anti-*S. typhi* lipopolysaccharide (LPS) monoclonal antibodies are immobilized on the test region 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* antibodies or anti-*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* LPS antibodies at the detection zone. The presence of one or more colored lines in the test region indicates a positive result, while the absence of such lines indicates a negative result. Excess colored particles are captured at the internal control region to indicate that a proper volume of specimen has been added and membrane wicking has occurred.

Precautions

- For professional laboratory *in-vitro* diagnostic use only.
- Do not use 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.

Materials

Materials provided

- Individually packed test devices
- Product Insert
- Sample dilution tubes with buffer and integrated dropper (1 per test cassette)

Materials required but not provided

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

Storage and Stability

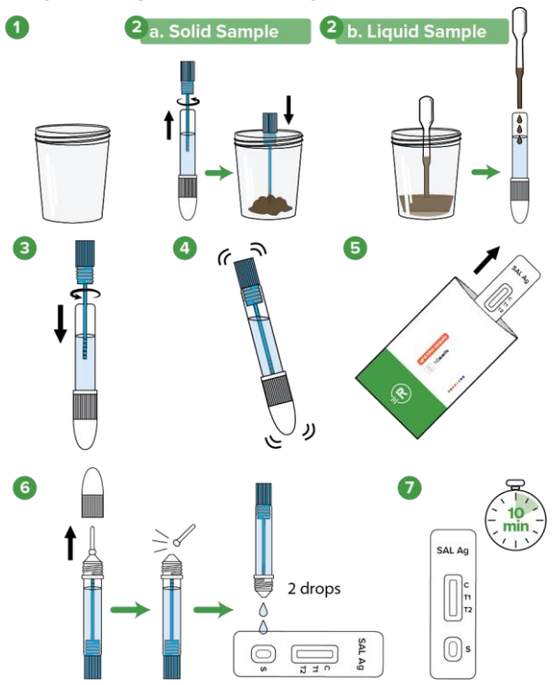
- The kit should be stored at 35.6-86°F (2-30°C) until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

Collection and Storage of Specimens

- Store the Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette at 35.6~86°F (2~30°C) when not in use.
- **DO NOT FREEZE.**
- The Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette is intended only for use with human fecal specimens.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.
- 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.
- Bring specimens to room temperature prior to testing.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 35.6-46.4°F (2-8°C) for up to 72 hours.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.

Test Procedure

Bring tests, specimens, buffer and/or controls to room temperature (59-86°F;15-30°C) before use.



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 2~8oC. For long-term storage, it is recommended to keep specimens below -4°F (-20°C).
2. **a. 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).
   b. For liquid specimens: Hold the dropper vertically, aspirate fecal specimens, and then transfer 3 drops

(approximately 100 µL) of the liquid specimen into the sample diluent tube.

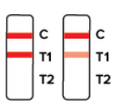
3. Place the applicator back into the tube and screw the cap tightly. Be careful not to break the tip of the dilution tube.
4. Shake the specimen collection tube to mix the specimen and the diluent buffer thoroughly.

Testing

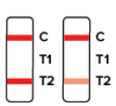
5. Remove the test device from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. To obtain a best result, the assay should be performed within one hour.
6. Using a piece of tissue paper, break the tip of the dilution tube. Hold the tube vertically 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.**
7. 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 specimens contained in the sample diluents tube. Collect 100 µL of supernatant, dispense into the specimen well (S) of a new test device and start afresh following the instructions mentioned above.

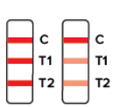
Results Interpretation



POSITIVE: *Salmonella Typhi*: Two colored lines appear. One colored line should be in the control region (C) and another colored line should be in the T1 region. A positive result in the T1 region indicates that *Salmonella Typhi* antigen was detected in the sample.

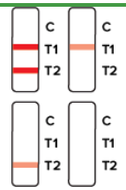


POSITIVE: *Salmonella Paratyphi*: Two colored lines appear. One colored line should be in the control region (C) and another colored line should be in the T2 region. A positive result in the T2 region indicates that *Salmonella Paratyphi* antigen was detected in the sample.



POSITIVE: *Salmonella Typhi* and *Salmonella Paratyphi:* Three colored lines appear. One colored line should be in the control region (C) and two colored lines should be in the T1 region and T2 region. A positive result in the T1 region and T2 region indicates that *Salmonella Typhi* antigen and *Salmonella Paratyphi* antigen was detected in the sample.

NEGATIVE: Only one colored line appears, in the control region (C). No apparent colored band appears in the test region (T).



INVALID: Control line fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test line regions (T1 or 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

- The Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette is for professional laboratory *in-vitro* diagnostic use, and should be used for the qualitative detection of *S. typhi* or *S. paratyphi* antigen only.
- 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.
- Failure to follow the Test Procedure and Result Interpretation may adversely affect test performance and/or invalidate the test result.
- 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.
- 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

Table 1. Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette vs. Culture

		Rapid Response® *Salmonella Typhi/Paratyphi* Test Cassette		
		+	-	**Total**
Culture	+	89	1	90
	-	1	123	124
	Total	90	124	214

Positive agreement: 89/90=98.9%
Negative agreement: 123/124=99.2%
Overall agreement: (89+123)/214=99.1%

Specificity

Cross reactivity with following organisms has been studied at 1.0 x 109 organisms/ml. The following organisms were found negative when tested with the Rapid Response® Salmonella Typhi/Paratyphi Test Cassette.

Staphylococcus aureus	Gardnerella vaginalis
Proteus mirabilis	Enterococcus faecium
Neisseria gonorrhea	Klebsiella pneumoniae
Pseudomonas aeruginosa	Acinetobacter calcoaceticus
Neisseria meningitidis	Hemophilus influenzae
Group B Streptococcus	Branhamella catarrhalis
Enterococcus faecalis	Escherichia coli
Helicobacter pylori	Candida albicans
Proteus vulgaris	Chlamydia trachomatis
Group C Streptococcus	Rotavirus

Bibliography

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

Consult instructions for use
Test per Kit
Do Not Reuse
Store between 35.6°F to 86°F
Use by
REF Catalogue #
LOT Lot Number
IVD For *in vitro* diagnostic use only

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