VITASSAY

Salmonella typhi+paratyphi

Rapid test for the simultaneous qualitative detection of Salmonella typhi and Salmonella paratyphi in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Salmonella typhi+paratyphi is a rapid, immunochromatographic, one step assay for the simultaneous qualitative detection of Salmonella typhi and Salmonella paratyphi in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of a Salmonella typhi infection and/or Salmonella paratyphi infection.

INTRODUCTION

Salmonella infections and enteric fever remain an important public health problem in many parts of the world, especially in developing countries.

The most severe form of Salmonella infection is typhoid fever caused by serovars adapted to a human host, such as Salmonella Typhi and Salmonella Paratyphi.

Salmonella enterica serotype Typhi and S. enterica serotype Paratyphi A, B and C cause typhoid and paratyphoid fevers. They are characterized by relatively long incubation periods (14 days on average) and are common in tropical regions, including South and Southeast Asia. Although imported cases in nontropical countries (e.g. the United States and Japan) have been documented among returning travelers, cases attributable to domestic origin in these countries are rare given the improvements in hygiene in the past decades.

These pathogens are acquired following ingestion of faecally-contaminated food or water or directly from asymptomatic carriers, and outbreaks are frequent. Important vehicles of transmission in some countries include shellfish taken from sewage-contaminated beds, raw fruits, vegetables fertilized by night soil and eaten raw, milk and milk products, or during preparation of food by hands.

PRINCIPLE

Vitassay Salmonella typhi+paratyphi is a qualitative immunochromatographic assay for the detection of salmonella typhi and salmonella paratyphi in human stool samples**.**

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Salmonella typhi.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Salmonella paratyphi.

During the process, the sample reacts with the antibodies against Salmonella typhi (strip A) and Salmonella paratyphi (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is Salmonella typhi positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A, and if the sample is Salmonella paratyphi positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip B. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for both strips).

The presence of these green lines (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Salmonella typhi+paratyphi**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Salmonella typhi+paratyphi** ▪ Instructions for use. ▪ **25** Vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

SPECIMEN COLLECTION

Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

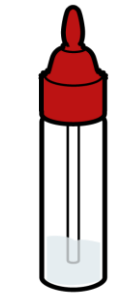
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

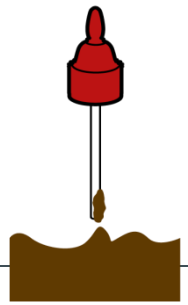
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, take 125 µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

1



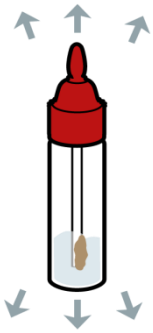
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



Put the sample into the vial, close the cap and shake.

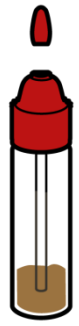
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Salmonella typhi+paratyphi** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter A – Salmonella typhi (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter B – Salmonella paratyphi (figure 6).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

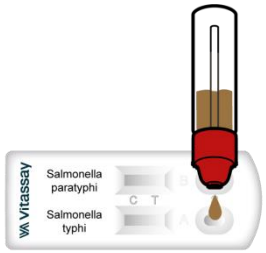
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



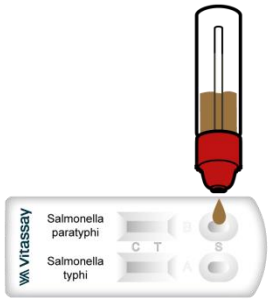
Cut the end of the cap.

5



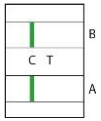
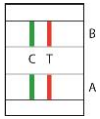
Dispense 4 drops in the circular window marked with the letter S to the strip A – Salmonella typhi.

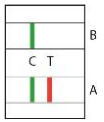
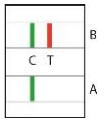
6



Dispense 4 drops in the circular window marked with the letter S to the strip B – Salmonella paratyphi.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Salmonella typhi	Strip B Salmonella paratyphi	INTERPRETATION
	Negative	Negative	There is no Salmonella typhi and/or Salmonella paratyphi presence. There is not infection caused by Salmonella typhi and/or paratyphi
	GREEN	GREEN	
	Positive	Positive	There is Salmonella typhi and Salmonella paratyphi presence. Infection caused by Salmonella typhi and paratyphi.
	GREEN-RED	GREEN-RED	

	**Positive**	**Negative**	There is Salmonella typhi presence. Infection caused by Salmonella typhi.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is Salmonella paratyphi presence. Infection caused by Salmonella paratyphi.
	GREEN	GREEN-RED	
ANY OTHER RESULTS			Invalid result, we Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are the main reasons of control line failure. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural controls are included in **Vitassay Salmonella typhi+paratyphi**. Green lines appearing in the in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Salmonella typhi+paratyphi** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of other samples different from human fecal samples has not been established.
- The quality of **Vitassay Salmonella typhi+paratyphi** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Salmonella typhi and/or salmonella paratyphi in human stool samples. A positive result should be followed up with additional laboratory techniques to confirm the results. A confirmed infection should only be made by a physician after the evaluation of all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the detection limit value. If symptoms or situation still persist, a Salmonella typhi and/or Salmonella paratyphi determination should be carried out on a sample from an enrichment culture.

EXPECTED VALUES

Gastroenteritis is commonly caused by nontyphoidal Salmonella such as Salmonella enterica serovar Typhimurium, serovar Enteritidis, serovar Stanley and serovar Weltevreden. The distribution of different Salmonella serovars varies among countries and regions. On the other hand, WHO estimated 17 million cases of typhoid fever annually , where Salmonella enterica serovar Typhi is the predominant organism isolated over the past decades, however, in some provinces in China and Pakistan, there is an increasing numbers of enteric fever cases caused by Salmonella Paratyphi A.

The disease remains a critical public health problem in developing countries. In 2000, it was estimated that over 21.6 million (incidence of 3.6 per 1000 population) of typhoid ocurrences world wide, resulting in 216000 deaths and that more than 90% of this morbidity and mortality ocurred in Asia.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with cultures was performed, comparing the results of **Vitassay Salmonella typhi + paratyphi** and another commercial test (Singlepath®Salmonella, Merck).

Results were as follows:

		Singlepath®Salmonella		
		Positive	**Negative**	**Total**
Vitassay Salmonella typhi + paratyphi	**Positive**	10	0	10
	Negative	0	25	25
Salmonella typhi	**Total**	10	25	35

		Singlepath®Salmonella		
		Positive	**Negative**	**Total**
Vitassay Salmonella typhi + paratyphi	**Positive**	10	0	10
	Negative	0	30	30
Salmonella paratyphi	**Total**	10	30	40

Vitassay Salmonella typhi + paratyphi vs Singlepath®Salmonella			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

Results showed that **Vitassay Salmonella typhi + paratyphi** has a high sensitivity and specificity to detect salmonella typhi and salmonella paratyphi.

Cross reactivity

No cross reactivity was detected against other gastrointestinal pathogens that are occasionally present in feces:

Campylobacter coli	*Listeria monocytogenes*	*Shigella flexneri*
Campylobacter jejuni	*Salmonella typhi (Tira B)*	*Shigella sonnei*
Clostridium difficile	*Salmonella paratyphi (Tira A)*	*Staphylococcus aureus*
E. coli O157: H7	*Shigella boydii*	*Yersinia enterocolitica*
Helicobacter pylori	*Shigella dysenteriae*	

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2. ABDULLAHI, MAS'UD. "Prevalent and Multiple drug resistance indexes of typhoidal and non-typhoidal salmonella isolated fronm stool samples of hospitalized subjects in Kano, North-West, Nigeria". Journal of Multidisciplinary Engineering Science and Technology, Vol. 2; Issue 12, December 2015, pp. 3360-3369.

3. TETSURO KOBAYASHI, SATOSHI KUTSUNA; KAYOKO HAYAKAWA; YASUYUKI KATO; NORIO OHMAGARI; HIDEKO URYU; RITSUKO YAMADA; NAOYUKI KASHIWA; TAKAHITO NEI; AKIHITO EHARA; REIKO TAKEI; NOBUAKI MORI; YASUHIRO YAMADA; TOMOMI HAYASAKA; NARITO KAGAWA; MOMOKO SUGAWARA; AI SUZAKI; YUNO TAKAHASHI; HIROYUKI NISHIYAMA; MASATOMO MORITA; HIDEMASA IZUMIYA; MAKOTO OHNISHI. "Outbreak of food-borne typhoid fever in Japan in 16 years". American Society of Tropical Medicine and Hygiene, 2016, vol. 94, no. 2, pp. 289-291.

4. MOHAMMAD ATIQUR RAHMAN. "Antimicrobial resistance patterns of Salmonella typhi isolated from stool culture". Chattagram Maa-O-Shishu Hospital Medical College Journal, Vol. 14, Issue 1, January 2015.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

Parque Tecnológico WALQA
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