VITASSAY

Salmonella typhi+paratyphi

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

IUE-7455031 Ed01 August 2023

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 non-tropical 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.
- Read the instructions for use carefully before using the test.
- Do not use the kit if the label sealing the outer carton is torn or if the bags are open or damaged on arrival.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- Do not reuse. This is a single-use device.
- Specimens should be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations. A new test should be used for each sample to avoid contamination errors.
- Material exposed to samples should also be considered potentially hazardous and should be handled in the same way as an infectious agent, following local/national regulations.
- Used material should be disposed of in an appropriate 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. These practices should include, but are not limited to, personal protective equipment (PPE), such as lab coat, surgical or appropriate mask or face shield, disposable gloves and eye protection. Take the necessary precautions during sample collection, transport, storage, handling and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink or smoke in the workplace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.

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- The visual interpretation of the results is done by coloured lines, the interpretation of the results should be done by a professional user without problems of visualisation and colour interpretation.
- A certificate of analysis can be provided on request (not included).

STORAGE AND STABILITY

The storage temperature of the kits should be 2-30ºC.

Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

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.	▪ PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer ▪ Micropipette (in case of liquid stool)

SPECIMEN COLLECTION

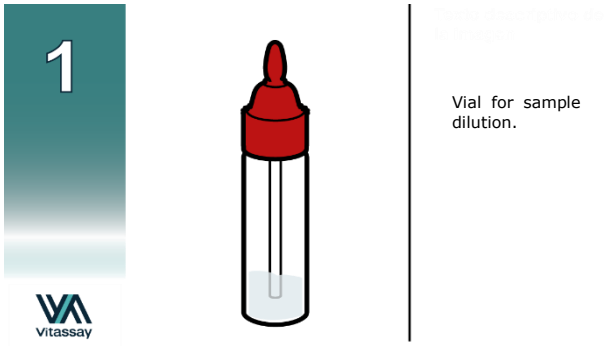
Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

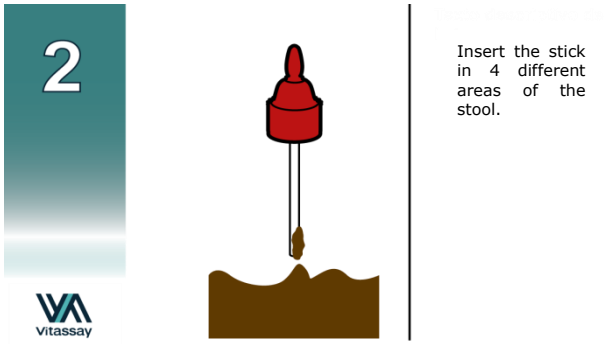
Samples can be stored in the refrigerator (2-8ºC) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC. Samples must be brought to room temperature before testing.

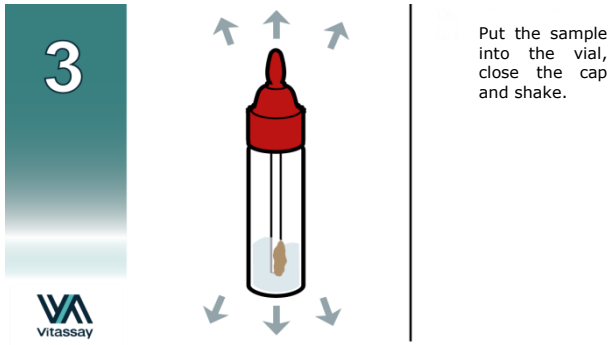
Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

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).





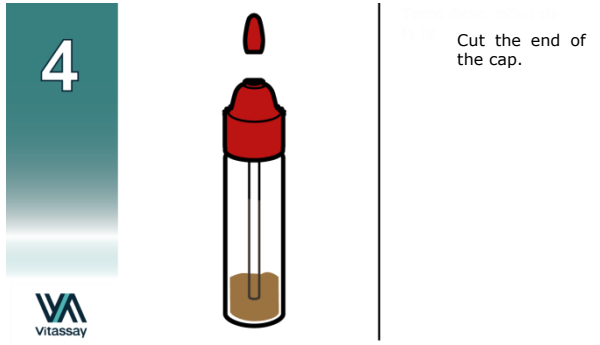


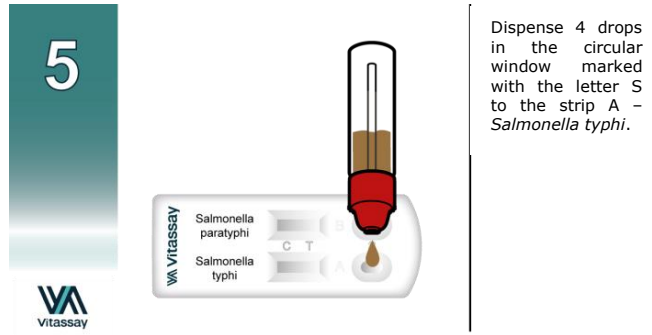
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) 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.





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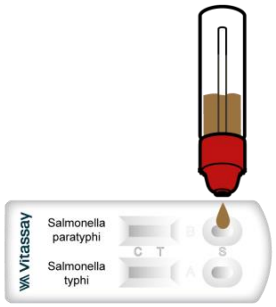
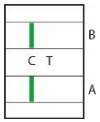
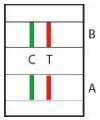
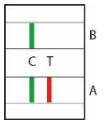
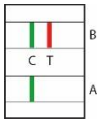


Dispense 4 drops in the circular window marked with the letter S to the strip B – *Salmonella paratyphi.*

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 recommend repeating the assay using the sample with another test. **Note**: Wrong procedural techniques, insufficient sample volume or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation 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

- 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 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.
- **Bloody stool samples** and/or **mucuos** stool samples can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the result

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 *Salmonella typhi*	Positive	10	0	10
	Negative	0	25	25
	Total	10	25	35

Table 1. Results of **Vitassay Salmonella typhi + paratyphi** compared to a commercial kit (Singlepath®Salmonella, Merck) for *S. typhi* detection.

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

Table 2. Results of **Vitassay Salmonella typhi + paratyphi** compared to a commercial kit (Singlepath®Salmonella, Merck) for *S. paratyphi* detection.

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

Table 3. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Salmonella typhi + paratyphi** compared to a commercial kit.

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

Analytical sensitivity

Vitassay Salmonella typhi + paratyphi has a Limit of Detection (LoD) for *Salmonella typhi* of 1.25x107 CFU/mL and for *S. paratyphi* of 3.125 x107 CFU/mL.

Cross reactivity

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

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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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
CE	CE Marking		

Changes control		
Nº Version	**changes**	**Date**
IUE-7455031 Ed00 August 2016	Original version	08/2016
IUE-7455031 Ed01 August 2023	Addition of Analytical sensitivity section. Format has been updated. Limitations sections has been updated. Transcription error in interpretation section has been corrected. Wording and grammatical changes have been implemented in Precautions, Limitations, Specimen collection, Storage and Stability. Material required but not included updated with minor changes.	25/08/2023

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