VITASSAY

Salmonella

Rapid test for the qualitative detection of *Salmonella* in human stool samples.

IUE-7355029 Ed03 October 2023

CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Salmonella is a rapid, immunochromatographic, one step assay for the qualitative detection of *Salmonella* in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of *Salmonella* infection (salmonellosis).

INTRODUCTION

Salmonella spp. and *Campylobacter spp.* (*Campylobacter coli and Campylobacter jejuni*) are recognized as the leading causes of bacterial gastroenteritis followed by Shigella spp. and Shiga toxin-encoding Escherichia coli (STEC).

Foodborne diarrheal outbreaks represent an important global health problem.

Salmonella is an enteroinvasive pathogen that most commonly causes self-limiting gastroenteritis.

Approximately 5% of all patients develop septicemia and the effect on children, elderly, and immunocompromised patients can lead to more serious complications, including death.

Salmonella enteritidis is considered the most common serovar in human infections, and most of these infections are associated with poultry products.

PRINCIPLE

Vitassay Salmonella is a qualitative immunochromatographic assay for the detection of *Salmonella* in human stool samples.

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

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

The presence of this green line (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.
- 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.
- Do not reuse. This is a single-use device.
- 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.
- All reagents included in the kit are approved for use with **Vitassay Salmonella** only. Do not mix or use the components with other batches of Vitassay. Do not use with reagents from other kits or commercial assays.
- 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.
- 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.

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

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Salmonella** ▪ 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

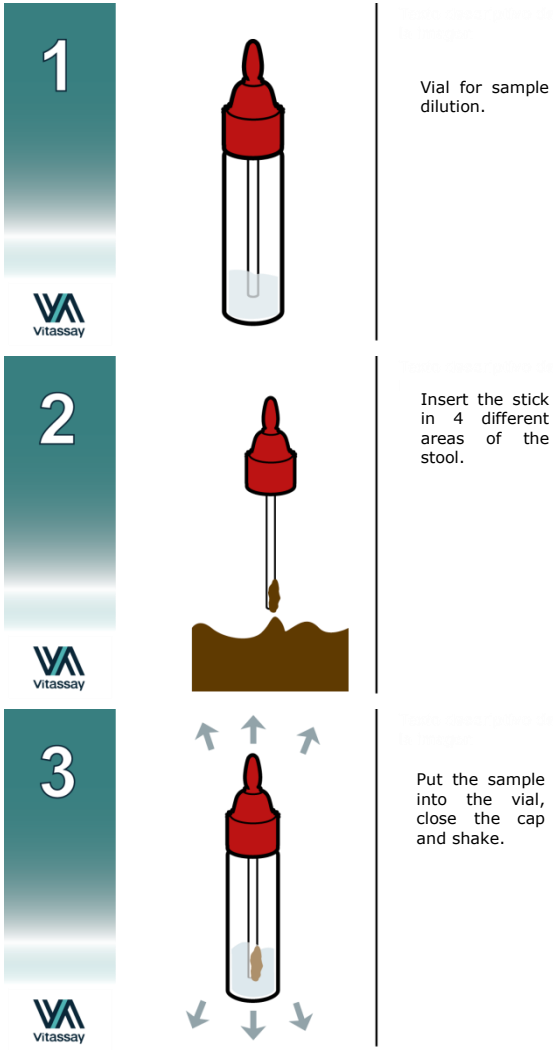
Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean containers.

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.

Freezing and defrosting cycles are not recommended, so ensure that only the amount needed is thawed. Homogenize 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. For solid stool, insert the stick in 4 different areas of the stool sample, (figure 2), and add it into the vial with diluent for the sample dilution. Not to exceed the stick´s screw to avoid wrong results. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
2. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

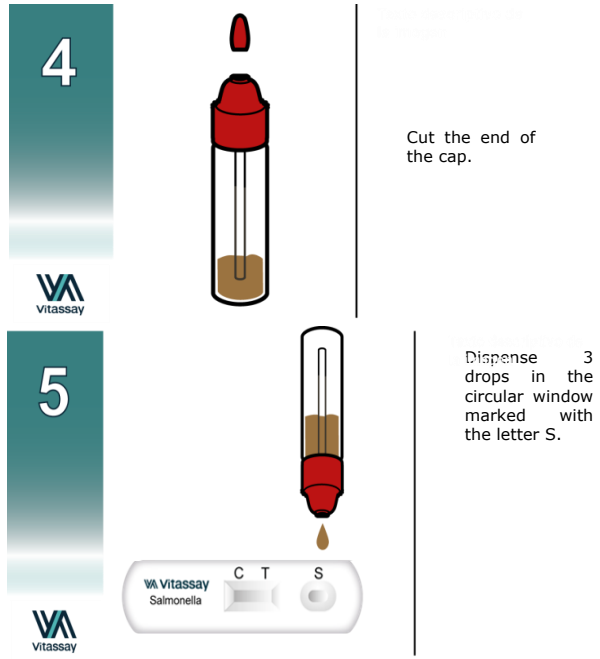


PROCEDURE

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 to obtain a good sample dilution.
2. Remove the **Vitassay Salmonella** 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 3 drops in the circular window marked with the letter S (figure 5).
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 (S) with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



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

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone (**C**).	There is no *Salmonella* presence. No infection caused by *Salmonella*.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears, (**test line T**)	There is presence of *Salmonella*. Possible *Salmonella* infection, which might mean abdominal pain, diarrhea, mild fever, chills, headache, nausea and vomiting, develops 12-72 hours (but occasionally as long as 7 days) after infection.
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques, deterioration of the reagents or insufficient specimen volume 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 control is included in **Vitassay Salmonella**. Green line appearing in the results window is an internal control, which confirms 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** depends on the quality of the sample; Proper faecal specimens must be obtained.
- ositive results determine the presence of Salmonella in human stool samples; however, a positive result should be cross-checked with other laboratory techniques to determine the strain and/or confirm the result. Infection should be confirmed by a specialist or qualified method, after evaluation of the clinical evidence and laboratory findings taking into account the correlation that may exist with all clinical observations.
- A negative result should not be considered as conclusive, it may be that the concentration of antigens in the stool sample is below the value the Limit of Detection. If symptoms or the situation persist, the determination of *Salmonella* should be repeated with the sample previously subjected to enrichment.
- **Bloody stool** samples and/or **mucous** 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

Food-borne illness in the United States accounts for an stimated 9.4 million cases of gastroenteritis, ≥50,000 hospitalizations, and 1,351 deaths each year.

Recent studies estimate that there are 80.3 million annual cases of *Salmnella* related diseases wordwide.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

The detection limit range for the different species are: $5{\cdot}10^7$ – $3.12{\cdot}10^6$ CFU/mL of inactivated *Salmonella enteritidis* antigen cells culture, $10{\cdot}10^6$ – $6.25{\cdot}10^5$ CFU/mL of inactivated *Salmonella typhimurium* antigen cells culture, $10{\cdot}10^7$ – $6.25{\cdot}10^6$ CFU/mL of inactivated *Salmonella typhi* antigen cells culture.

Clinical sensitivity and specificity

On the one hand, a first analysis was performed comparing the Vitassay Salmonella kit with a commercial kit for the detection of Salmonella antigens in feces, considered as a reference method.

The results obtained are shown below:

		Reference method		
		Positive	**Negative**	**Total**
Vitassay Salmonella	**Positive**	10	1	11
	Negative	0	33	33
	Total	10	34	44

Table 1. Results of **Vitassay Salmonella** compared to the reference ethod

Vitassay Salmonella vs comercial kit			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>97%	>91%	>99%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Salmonella** vs the reference method.

On the other hand, an evaluation was carried out with remnants of faecal samples using **Vitassay Salmonella**. The results were compared with the characterisation of the samples using a commercial multiplex (real-time PCR) for the detection and differentiation of Salmonella, Campylobacter and entercolytic Yersisin, which was considered the reference method for this study.

The results are shown below:

		Reference method		
		Positive	**Negative**	**Total**
Vitassay Salmonella	**Positive**	47	2	49
	Negative	15	103	118
	Total	62	105	167

Table 3. Results of Vitassay Salmonella compared to a commercial qPCR kit

Vitassay Salmonella vs Reference method		
	Mean Value	**95% confidence interval**
Sensitivity	75.8%	63.3-85.8%
Specificity	98.1%	93.5-99.8%
PPV	95.9%	86.0-99.5%
NPV	87.3%	79.9-92.7%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Salmonella** compared to a commercial qPCR kit.

The results showed that **Vitassay Salmonella** has a high sensitivity and specificity to detect *Salmonella*.

Cross reactivity

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

Adenovirus	*Escherichia coli O:026*	*Norovirus GII*
Astrovirus	*Escherichia coli O157*	*Rotavirus*
Calprotectin	*Entamoeba dispar*	*Shigella boydii*
Campylobacter coli	*Entamoeba hystolitica*	*Shigella dysenterae*
Campylobacter jejuni	*Giardia lamblia*	*Shigella flexneri*
Clostridium difficile	*Helicobacter pylori*	*Shigella sonnei*
Clostridium difficile Toxin A	*Hemoglobin*	*Sreptococcus pneumoniae*
Clostridium difficile Toxin B	*Lactoferrin*	*Sreptococcus pyogenes*
Clostridium perfringens	*Legionella*	*Transferrin*
Cryptosporidium	*Listeria monocytogenes*	*Yersinia enterocolitica O:3*
Escherichia coli O:111	*Norovirus GI*	*Yersinia enterocolitica O:9*

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

REFERENCES

1. BLAKE W. BUCHAN; WENDY J. OLSON; MICHAEL PEZEWSKI; MARIO J. NOVICKI; TIMOTHY S. UPHOFF; LAKSHMI CHANDRAMOHAN; PAULA REVELL; NATHAN A. LEDEBOER. "Clinical evaluation of a Real-Tie PCR assay for identification of Salmonella, Shigella, Campylobacter (campylobacter jejuni and C. coli), and Shiga toxin-producing Escherichia coli isolates in stool specimens". Journal of Clinical Microbiology, December 2013, Volume 51, Number 12, pp. 4001-4007.

2. MARIA REGINA PIRES CARNEIRO; PEDRO HERNAN CABELLO; RICARDO LUIZ CAVALCANTI ALBUQUERQUE-JUNIOR; SONA JAIN; ALEXANDRE LUNA CANDIDO. "Characterization of a foodborne outbreak caused by Salmonella Enteritidis in Aracaju, State of Sergipe, Brazil". Revista da Sociedade Brasileira de Medicina Tropical 48(3):334-337, May-Jun, 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**	**D**ate
IUE-7355029 Ed02 August 2023	Incorporation of a new evaluation. The limitations section has been updated. Grammatical and editorial changes have been made to Precautions, Limitations and editorial changes have been made to Precautions, Limitations, Sample Collection, Preservation and Stability, and Interpretation of Results, Preservation and Stability, and Interpretation of results. Material required, but not included updated with minor changes.	25/08/2023
IUE-7355029 Ed03 October 2023	Homepage error was corrected: it stated June instead of August. Corrected according to the current date. Correction of IFU name in history change	05/10/2023

Vitassay