VITASSAY

E. coli

Rapid test for the qualitative detection of E. coli O157:H7 in human stool samples.

IUE-7355026 Ed01 August 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay E. coli is a rapid one step immunochromatographic assay for the qualitative detection of Escherichia coli O157:H7 (E. coli O157:H7) in human stool samples.

Simple, non-invasive and a highly sensitive screening assay to make a presumptive diagnosis of Escherichia coli O157:H7 infection.

INTRODUCTION

E. coli O157:H7 infections can range in severity from asymptomatic carriage to bloody diarrhea and severe abdominal cramping, hemolytic uremic syndrome (HUS), and death. The principal reservoirs of E. coli O157:H7 are ruminant animals (e.g., cattle, sheep, goats, and deer). Infections are acquired by consumption of fecal contaminated water or food (especially meat or produce), via person-to-person spread, and from contact with colonized animal or their environments.

Human infection occurs 3-4 days after bacteria are ingested; symptoms include diarrhea, vomiting, stomach cramps, and a low grade fever lasting for 5-7 days.

PRINCIPLE

Vitassay E. coli is a qualitative immunochromatographic assay for the determination of Escherichia coli O157:H7 in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against E. coli O157:H7.

During the process, the sample reacts with the antibodies against E. coli O157:H7, 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.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- 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 the samples should also be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations.
- Do not reuse. This is a single-use device.
- Used material must 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 E. coli** 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.

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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 E. coli** ▪ 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

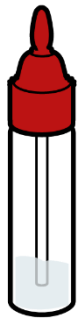
Collet sufficient quantity of feces: 1-2g or mL for liquid samples. Stool should be collected in clean and dry containers.

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.

SPECIMEN PREPARATION

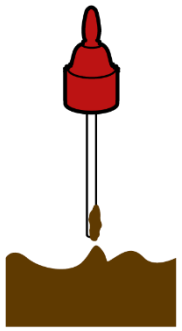
1. Remove the cap of vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for sample dilution. For liquid stool, take 125µL 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



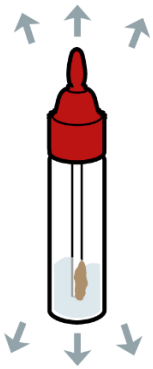
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

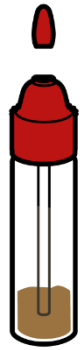
PROCEDURE

Allow the test, stool sample, controls (if applicable) 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 E. coli** 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 S (figure 5).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

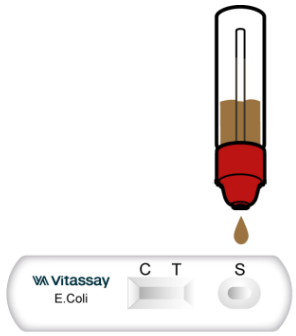
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



Cut the end of the cap.

5



Dispense 4 drops in the circular window marked with the letter S.

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one **green** line in the control zone **(C)**.	There is no *E. coli O157:H7* presence. No infection caused by *E. coli O157:H7.*
	**POSITIVE** In addition to the **green** line (**control line C**), a **red** line appears (**test line T**)	There is presence of *E. coli O157:H7.* Possible *E. coli* infection, presents with a wide spectrum of clinical manifestation, including asymptomatic carriage, non-bloody diarrhea, hae-morrhagic colitis, the hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP).

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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 control is included in **Vitassay E. coli**. 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 E. coli** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of *E. coli O157:H7* in fecal samples; nevertheless, a positive result should be followed up with additional laboratory techniques (biochemical method or by PCR) to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated 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 antigens is lower than the detection limit. If symptoms or situation still persist, an E. coli determination should be carried out, on a sample from an enrichment culture.
- **Bloody stool samples** and/or **mucous** stool samples can cause non-specific reactions in the test. These types of samples whose result is positive should be followed up with other techniques of diagnosis to confirm the result.
-

EXPECTED VALUES

Escherichia coli O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains are an important cause of bacterial gastrointestinal ilness in the United States. Illness can be severe, especially in young children or the elderly, and hemolytic-uremic syndrome (HUS) occurs in 4%-13% of patients. E. coli O157 infection is the most common cause of HUS in children.

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is the causal agent for more than 96000 cases of diarrheal illness and 3200 hospitalizations annually in the United States.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit value established of **Vitassay E. coli** is 1.87x104CFU/mL.

Clinical sensitivity and specificity

Vitassay E. coli was evaluated to determine sensibility in selective enrichment culture and samples, specificity with producer organisms of Shiga toxins, non-Shiga toxins producers and other Enterobacteriaceae species in the Reference Laboratory for Escherichia coli (LREC), Universidad Santiago de Compostela, Lugo (SPAIN).

14 STEC strains (O157:H7 antigen), 4 Non STEC strains (O157), 9 STEC strains (non O157), 4 other Enterobacteriaceae spp.

Results were as follows:

		Culture			
		Positive STEC (O157 antigen)	Negative Non STEC (O157)	Negative Non O157	Total
Vitassay E. coli	Positive	14	4	2	20
	Negative	0	0	11	11
	Total	14	4	13	31

Table 1. Results of **Vitassay E. Coli** compared to the reference method used at the hospital (culture).

Vitassay E. coli vs Cultivo			
Sensitivity	Specificity	PPV	NPV
>99%	85%	70%	>99%

Table 2. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay E. Coli** kit in comparison the reference method used at the hospital (culture).

The results showed that **Vitassay E. coli** has a high sensitivity and specificity to detect *E. coli O157:H7*.

Cross reactivity

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

Campylobacter coli	*E. coli O171:H2*	*Salmonella typhi*
Campylobacter jejuni	*E. coli O174:H8*	*Salmonella typhimurium*
Citobacter freundii	*Klebriella pneumoniae*	*Shigella boydii*
Clostridium difficile	*Helicobacter pylori*	*Shigella dysenteriae*
E. coli O22:H8	*Listeria monocytogenes*	*Shigella flexneri*
E. coli O91:H-	*Morganelle morganii*	*Shigella sonnei*
E. coli O103:H2	*Proteus mirabilis*	*Staphylococcus aureus*
E. coli O111:H21	*Salmonella enteritidis*	*Yersinia enterocolitica*
E. coli O145:H-	*Salmonella paratyphi*	

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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**
IU-7355001 Ed01 August 2023	Change of format. Addition of a new evaluation. 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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