VITASSAY

E. coli

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

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CE IVD EN

Vitassay

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.
- 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. Single use device.
- 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 E. coli**. 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) on the sealed pouch.

The test is stable until the expiration date printed.

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 E. coli** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

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/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.

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

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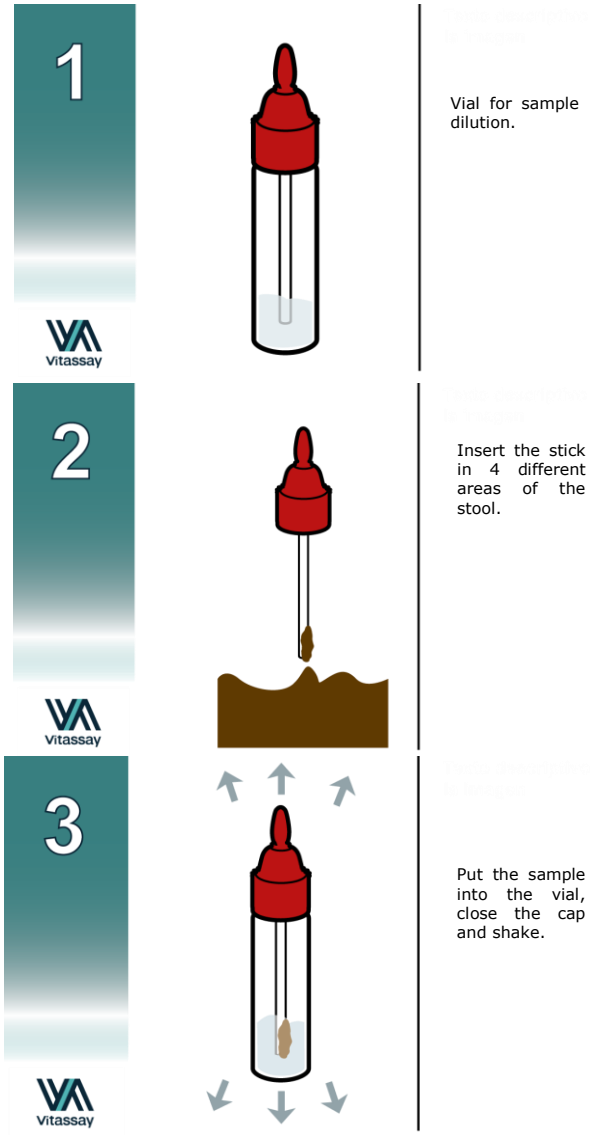
diluent for sample dilution. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.

3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

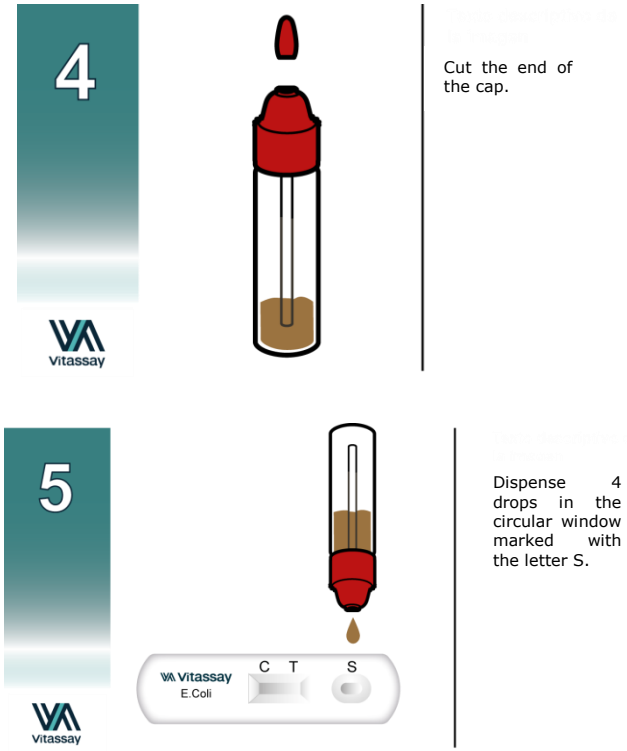


PROCEDURE

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 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.



INTERPRETATION OF THE RESULTS

C T	**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.
C T	**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. E. coli infection, presents with a wide spectrum of clinical manifestation, including asymptomatic carriage, non-bloody diarrhea, hemorrhagic colitis, the hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP).
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques 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

- **Vitassay E. coli** 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 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

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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.

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

Clinical sensitivity and specificity

Vitassay E. coli was evaluated to determine sensibility in selective enrichment culture and samples, specificity with producers 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

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

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

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