VITASSAY

EHEC VT1+VT2

Rapid test for the simultaneous qualitative detection of Verotoxins 1 and 2 (VT1 and VT2) produced by E. coli in human stool samples.

IUE-7455027 Ed01 August 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay EHEC VT1+VT2 is a rapid, immunoassay, one step for the simultaneous qualitative detection of Verotoxins 1 and 2 (VT1 and VT2) produced by *E. coli* in human stool.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of enterohaemorrhagic *E. coli* infection (*EHEC*).

INTRODUCTION

Diarrheal diseases are a leading cause of childhood morbidity and mortality in developing countries, accounting for around 2 million deaths annually. Among the aetiological agents of diarrhea in developing countries, diarrhoeagenic *Escherichia coli* (*DEC*) is an important agent, mostly in children younger than 5 years, and represents a major public health problem in these áreas. Six different types of *DEC* have been identified: *enteroaggregative E. coli* (*EAEC*), *enterohaemorrhagic E. coli* (*EHEC*), *enterotoxigenic E. coli* (*ETEC*) and diffusely adherent *E. coli*.

EHEC produces *Shiga* toxin encoded by stx1 (VT1) or stx2 (VT2) and is the most important recently emerged group of food-borne pathogens. It is a major cause of gastroenteritis that may be complicated by haemorrhagic colitis or haemolytic-uremic syndrome, which is the main cause of acute renal failure in children.

These toxins (VT1 and VT2) are the major virulence factors contributing to *EHEC* pathogenicity.

There are four main transmission routes identified through which *EHEC* can be transmitted to humans: foodborne transmission; waterborne transmission; person-to-person transmission; and direct contact with animals.

PRINCIPLE

Vitassay EHEC VT1+VT2 is a qualitative immunochromatographic assay for the detection of Verotoxin 1 and 2 (VT1 and VT2) produced by *E. coli* in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against VT1.
Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against VT2.

During the process, the sample reacts with the antibodies against EHEC VT1 (strip A) and EHEC VT2 (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is EHEC VT1 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 EHEC VT2 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 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 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 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 EHEC VT1+VT2**. Do not use any other commercial kit component nor with components of other batches.
- 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 EHEC VT1+VT2** ▪ Instructions for use. ▪ **25** vials with diluent for the dilution sample.	▪ 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.

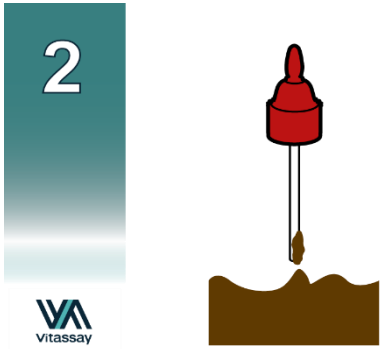
Samples can be stored in the refrigerator (2-8ºC) for 24 hours. For longer storage, maximum 2 weeks, the specimen must be kept frozen at -20ºC. Samples must be brought to room temperature before testing.

Freezing and thawing cycles are not recommended, therefore, thaw only the necessary amount of sample to perform the test. Homogenize the sample vigorously before preparation.

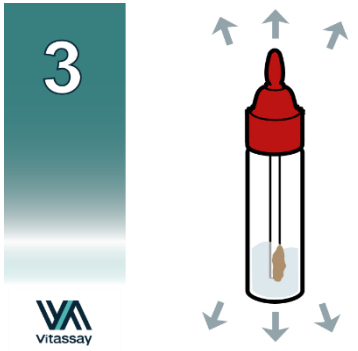
SPECIMEN PREPARATION

1. Remove the cap of the stool collection tube (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 (figure 2), and add it into the collection tube. For liquid stool, take 125 µL of the sample using a micropipette and transfer it into the collection tube.
3. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion (figure 3).

Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



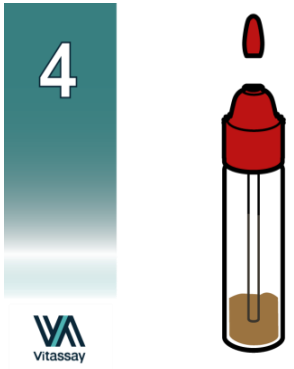
Put the sample into the vial, close the cap and shake.

PROCEDURE

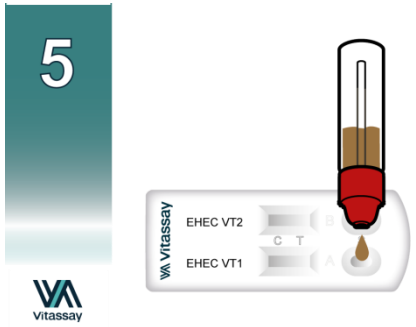
Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial vigorously to obtain a good sample dilution.
2. Remove the **Vitassay EHEC VT1+VT2** 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 – VT1 (figure 5) and 4 drops, using the sample vial, in the circular window marked with the letter B – VT2 (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.



Cut the end of the cap.



Dispense 4 drops in the circular window marked with the letter A – VT1.

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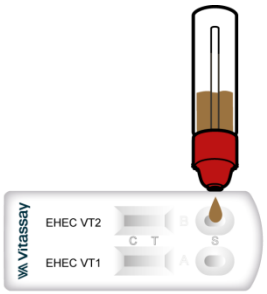
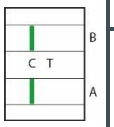
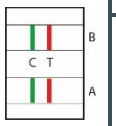
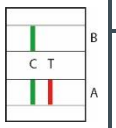
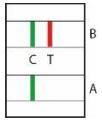


Dispense 4 drops in the circular window marked with the letter B – VT2.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A EHEC VT1	Strip B EHEC VT2	INTERPRETATION
	**Negative**	**Negative**	There is no VT1 or VT2 of *E. coli* presence. No infection caused by *EHEC*.
	GREEN	GREEN	
	**Positive**	**Positive**	There is VT1 and VT2 of *E. coli* presence. Possible infection caused by *EHEC*.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is VT1 of *E. coli* presence. Possible infection caused by *EHEC*.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is VT2 of E. coli presence. Possible infection caused by EHEC.
	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 the main reasons of 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 EHEC VT1+VT2**. Green lines appearing 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.
- After one week of infection, the number of bacteria in feces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the unset of symptoms.
- The quality of **Vitassay EHEC VT1+VT2** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of VT1 and/or VT2 of *E. coli* in human stool samples. A positive result should be followed up with additional laboratory techniques to confirm the results and/or determine the strain.
- 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, an EHEC determination should be carried out on a sample from an enrichment culture.
- Verotoxin 1 (Shiga-like toxin 1) produced by *EHEC* is very similar to one produced by *Shigella dysenteriae* type 1 (Shiga toxin). Verotoxin 1 differs from true Shiga toxin by one to seven aminoacids differences. **Vitassay EHEC VT1+VT2** may give a positive result with Shiga toxin produced by *Shigella dysenteriae* type 1. EHEC and *Shigella dysenteriae* type 1 determination should be carried out on selective culture and with biochemical analysis.
- **Bloody** stool samples and/or **mucuos faeces** may cause non-specific reactions in the test. Such positive samples should be contrasted with other diagnostic techniques to confirm the result.

EXPECTED VALUES

The epidemiological significance of diarrhoeagenic *Escherichia coli* types in childhood diarrhoea varies with geographical area. As expected, it has become clear that there are important regional differences in the prevalence and other epidemiological features of these pathogens.

In Japan, EHEC has caused 3000 – 4000 cases of illness annually in the last decade.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation was performed using **Vitassay EHEC VT1+VT2** and these results were compared with a commercial test (Shiga Toxin Quik Chek, TechLab®) for Verotoxin 1 and 2.

Results were as follows:

		Shiga Toxin Quik Chek, TecLab®		
		Positive	Negative	Total
Vitassay EHEC VT1+VT2	Positive	4	0	4
	Negative	0	30	30
	Total	4	30	34

Table 1. Results of **Vitassay EHEC VT1+VT2** compared to a commercial kit.

Vitassay EHEC VT1+VT2 vs Shiga Toxin Quik Chek, TechLab®			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay EHEC VT1+VT2** compared to a commercial kit.

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Results showed that **Vitassay EHEC VT1+VT2** has a high sensitivity and specificity to detect *EHEC*.

Cross reactivity

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

Campylobacter coli	*Listeria monocytogenes*	*Salmonella typhi*
Campylobacter jejuni	*Morganelle morganii*	*Salmonella typhimurium*
Citobacter freundii	*Proteus mirabilis*	*Staphylococcus aureus*
Clostridium difficile	*Salmonella enteritidis*	*Yersinia enterocolitica*
Klebsiella pneumoniae	*Salmonella paratyphi*	

REFERENCES

1. FAKHRI HAGHI; HABIB ZEIGHAMI; FAHIMEH HAJIAHMADI; HAKIMEH KHOSHVAGHT; MARZIYEH BAYAT. "Frequency and antimicrobial resistance of diarrhoeagenic Escherichia coli from young children in Iran". Journal of Medical Microbiology (2014), 63, 427-432.

2. SHIGEKO UEDA; MIKI IWASE; MANAMI YAMAGUCHI. "Evaluation of the Immunochromatographic Device for the Detection of Verotoxins in Cultures of Food Materials". Biocontrol Science, 2014, Vol. 19, No. 4, 205-208.

3. M.A. JORIS; D. VANROMPAY; K. VERSTRAETE; K. DE REU; L. DE ZUTTER. "Enterohemorrhagic Escherichia coli with particular attention to the German outbreak strain O104: H4". Vlaams Diergeneeskundig Tijdschrift, 2012, 81, pp. 3-10.

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-7455027 Ed00 April 2016	Original version	04/2016
IUE-7455027 Ed01 August 2013	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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