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.

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CE IVD EN

Vitassay

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 (EPEC), 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 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 EHEC VT1+VT2**. 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).
The test is stable until the expiration date printed on the sealed pouch.
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 EHEC VT1+VT2** ▪ Instructions for use. ▪ **25** vials with diluent for the dilution sample.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

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SPECIMEN COLLECTION

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

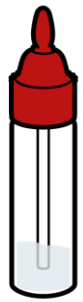
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 24 hours. For longer storage, maximum 2 weeks, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

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 (See illustrations)

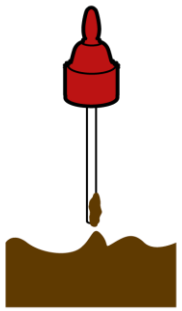
1. Remove the cap of the stool collection tube (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample (figure 2), and add it into the collection tube. For liquid stool, take 125 µL of the sample using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

1



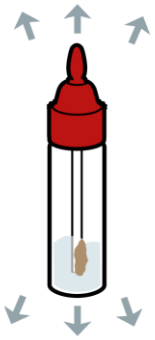
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

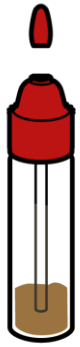
PROCEDURE

Allow the test, stool sample, controls 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 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.

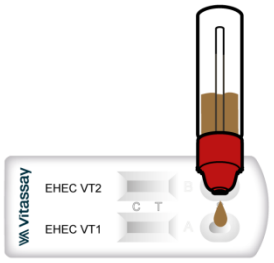
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



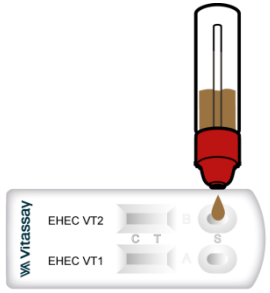
Cut the end of the cap.

5



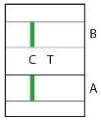
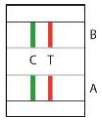
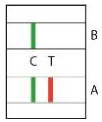
Dispense 4 drops in the circular window marked with the letter A – VT1.

6



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. Infection caused by EHEC.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is VT1 of E. coli presence. Infection caused by EHEC.
	GREEN-RED	GREEN	

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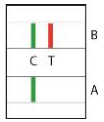
	Negative	Positive	
	GREEN	GREEN-RED	There is VT2 of E. coli presence. Infection caused by EHEC.
ANY OTHER RESULTS			Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are the main reasons of control 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

- **Vitassay EHEC VT1+VT2** 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.
- Only fresh or fresh-frozen unpreserved stool samples can be tested.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of others samples different from human fecal 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. 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.

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

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

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 feces:

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

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