VITASSAY

Enterovirus

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

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Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

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

Simple, non-invasive and highly sensitivity screening assay to make a presumptive Enterovirus infection.

INTRODUCTION

The Enteroviruses are a group of small RNA viruses, picornavirus, found mainly in the human intestine, usually in asymptomatic individuals, the susceptible populations being children.

The human enteroviruses belong to the genus *Enterovirus* and *Picornaviridae* family. These agents infect millions of people worldwide each year, resulting in a wide variety of clinical conditions ranging from unapparent infection, undifferentiated fevers, common cold to serious diseases such as aseptic meningitis, hand-foot-mouth disease, acute haemorrhagic conjunctivitis, myocarditis, encephalitis, and paralytic poliomyelitis. Children are more susceptible to infection and transmission occurs either by the faecal-oral or respiratory tract. The virus can be excreted in the faeces for several weeks.

Infections are transmitted mainly through the faecal-oral and oral-oral route but also through direct contact with secretions from ophthalmic and dermal lesions. Contact with water, food, and soil contaminated with infected faeces may cause faecal-oral transmission.

Poliovirus, coxsackievirus, echovirus, newer enterovirus and rhinovirus represent major pathogenic species of humans in the enterovirus genus and together present about 200 serotypes.

The main infection sources are typically ill patients and asymptomatic persons carrying the virus.

PRINCIPLE

Vitassay Enterovirus is a qualitative immunochromatographic assay for the detection of Enterovirus (VP1 peptide) in human stool samples.

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

During the process, the sample reacts with the antibodies against Enterovirus, 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.
- 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.
- Tests and 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.
- Components provided in the kit are approved for use with the **Vitassay Enterovirus**. Do not use any other commercial kit component or components from other batched.
- 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.

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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 Enterovirus** ▪ 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

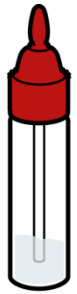
Collect sufficient quantity of faeces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

Samples can be stored in the refrigerator (2-8ºC) for 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

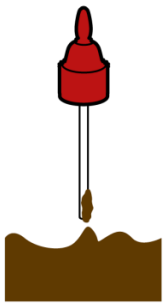
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution.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



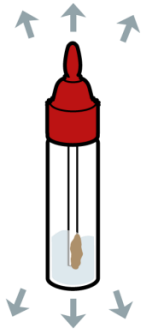
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

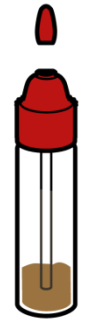
PROCEDURE

Allow the test, stool sample, controls 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 Enterovirus** 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.

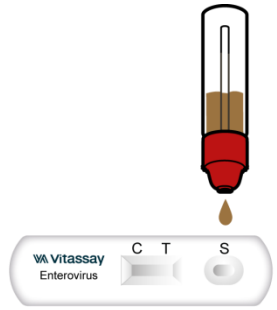
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



Cut the end of the cap.

5



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

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INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C)**	There is no Enterovirus presence. No apparent infection caused by Enterovirus.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears, **test line(T)**	There is Enterovirus presence. Probable viral infection caused by Enterovirus.
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-coloured 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 Enterovirus**. 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 Enterovirus** depends on the quality of the sample. Proper faecal specimens must be obtained.
- After one week of infection, the presence of virus shed in faeces decreases considerably, so a lower concentration in the sample is probable. It is advisable to take the stool sample before the onset of symptoms to prevent the spread of the pathogen, or 24/48h after the onset of the first symptoms.
- Positive results determine the presence of Norovirus (GI and/or GII) in faecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods or PCR) to confirm the results.
- A positive result may appear in the case of recently vaccinated patients. For example, if the patient has been vaccinated against *Poliovirus*.
- Negative results should not be considered conclusive; it is possible that the concentration of antigens in the faecal sample is below the detection limit value. If symptoms or the situation persists, an enterovirus determination with another technique (e.g., PCR) should be performed.
- **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

The incidence and severity of infections among infants are inversely related to their age, being more common in neonates and preterm infants.

Human Enterovirus type 71 (EV71) has emerged as a major cause of viral encephalitis in children worldwide.

EV71 outbreaks are reported throughout the world but have been especially severe in the Asia-Pacific region.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with faecal samples was performed using **Vitassay Enterovirus** and these results were compared with a commercial test (IDEIA Enterovirus assay, Dako and IMAGEN™ Enterovirus, Oxoid).

Results were as follows:

		IDEIA Enterovirus assay and IMAGEN™ Enterovirus		
		Positive	**Negative**	**Total**
Vitassay Enterovirus	**Positive**	3	0	3
	Negative	0	32	32
	Total	3	32	35

Table 1. Results of **Vitassay Enterovirus** compared to a commercial immunochromatographic kit.

Vitassay Enterovirus vs IDEIA Enterovirus assay and IMAGEN™ Enterovirus			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

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

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

Cross reactivity

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

Astrovirus	*Helicobacter pylori*	*Shigella sonnei*
Campylobacter coli	Norovirus	*Shigella flexneri*
Campylobacter jejuni	Rotavirus	*Shigella dysenteriae*
Clostridium difficile	*Listeria monocytogenes*	*Shigella boydii*
Cryptosporidium parvum	*Salmonella enteritidis*	*Staphylococcus aureus*
Escherichia coli O157:H7	*Salmonella typhi*	*Yersinia enterocolítica*
Entamoeba histolytica	*Salmonella typhimurium*	
Giardia lamblia	*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**
IUE-7355018 Ed00 April 2016	Original version	04/2016
IUE-7355018 Ed01 October 2023	The format has been updated. A transcription error in the interpretation section has been corrected. The limitations section has been updated. Grammatical and editorial changes have been made to the Precautions, Limitations, Sample Collection, Storage and Stability sections. Required but not included material updated with minor changes. The wording has been changed under Interpretation of results.	03/10/2023

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