VITASSAY

Enterovirus

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

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CE IVD EN

Vitassay

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 hemorrhagic conjunctivitis, myocarditis, encephalitis and paralytic poliomyelitis. Children are more susceptible to infection and transmission occurs either by the fecal-oral or respiratory tract. The virus can be excreted in the feces for several weeks.

Infections are transmitted mainly through the fecal-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 feces may cause fecal-oral transmission.

Poliovirus, coxsackievirus, achinovirus, 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.
- 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 Enterovirus**. 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 Enterovirus** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

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 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.

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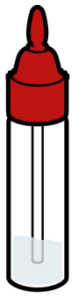
SPECIMEN PREPARATION

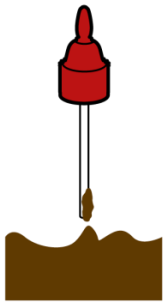
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution.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



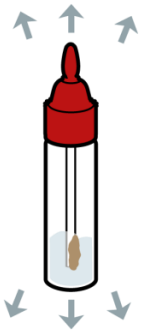
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

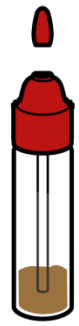
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC/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 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.

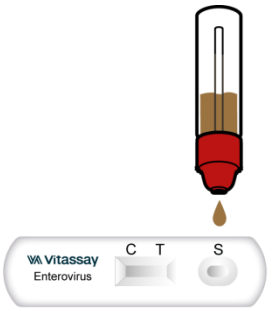
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



Cut the end of the cap.

5



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

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C)**	There is no enterovirus presence. No 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. 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 or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation still 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 Enterovirus**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

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LIMITATIONS

- **Vitassay Enterovirus** 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.
- After one month of infection, the number of viruses in feces is decreasing, making the sample less reactive. Stool samples could be collected previously to the onset of symptoms or also at 24-48 hours.
- If the patient has been recently vaccinated (for example against Poliovirus), it could appear a positive result.
- 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 fecal specimens must be obtained.
- Positive results determine the presence of Enterovirus in fecal samples. A positive result should be followed up with diagnostic procedures to confirm the results and the species. 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 value. If symptoms or situation still persist, an Enterovirus determination should be carried out with another technique (for example microscopy, biochemical method).

EXPECTED VALUES

The incidence and severity of enterovirus 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 fecal 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

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

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 feces:

Astrovirus	*Helicobacter pylori*	*Shigella boydii*
Campylobacter coli	*Listeria monocytogenes*	*Shigella dysenteriae*
Campylobacter jejuni	*Norovirus*	*Shigella flexneri*
Clostridium difficile	*Rotavirus*	*Shigella sonnei*
Cryptosporidium parvum	*Salmonella enteritidis*	*Staphylococcus aureus*
Entamoeba hystolitica	*Salmonella paratyphi*	*Yersinia enterocolítica*
Escherichia coli O157:H7	*Salmonella typhimurium*	
Giardia lamblia	*Salmonella typhi*	

REFERENCES

1. JIAN CAI; HUAKUN LV; JUNFEN LIN; ZHIPING CHEN; CHUNFU FANG; JIANKANG HAN. "Enterovirus Infections in Children attending two outpatient clinics in Zhejiang Province, China". J. Med. Virol. 86:1602-1608, 2014

2. DIOCRECIANO M. BERO; NILSA DE DEUS; ELIANE V. DA COSTA; FERNANDA M. BURLANDY; ILESH V. JANI; EDSON E. DA SILVA. "Natural circulation of human enterovirus in Maputo city, Mozambique". African Jorunal of Microbiology Research, Vol. 9(21), pp. 1419-1423, 27 May, 2015.

3. HUA-BING ZHAO; GUANG-YA YIN; GUO-PING ZHAO; AI-HUA HUANG; JUN-HONG WANG; SHUANG-FENG YANG; HONG-SHENG GAO; WEI-JUN KANG. "Development of Loop-Mediated Isothermal amplification (LAMP) for Universal detection of Enteroviruses". Indian Journal of Microbiology (Jan-Mar 2014) 54(1):80-86.

4. C. DURGA RAO; P.P. MAIYA; M. ANANDA BABU. "Non-diarrhoeal increased frequency of bowel movements (IFoBM-ND): enterovirus association with the symptoms in children". BMJ Open Gastro 2014; 1:e000011.

5. SUHAIL AHMAD; AJMAL DALWAI; WIDAD AL-NAKIB. "Frequency of Enterovirus Detection in Blood samples of neonates admitted to Hospital with sepsis-like illness in Kuwait". Journal of Medical Virology 85:1280-1285 (2013)

6. PENG-NIEN HUANG; SHIN-RU SHIH. "Update on enterovirus 71 infection". Current opinion in Virology, vol 5, april 2014, pages 98-104.

7. PAVEL PLEVKA; RUSHIKA PERERA; MOH LAN YAP; JANE CARDOSA; RICHARD J. KUHN; MICHAEL g. ROSSMANN. "Structure of human enterovirus 71 in complex with a capsid-binding inhibitor". PNAS, April 2, 32013, vol. 110, no.14, pp. 5463-5467.

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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