VITASSAY

Rotavirus

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

IUE-7355008 Ed01 October 2023

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Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

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

Simple, non-invasive and a highly sensitive screening assay to make a presumptive diagnosis of Rotavirus infection.

INTRODUCTION

Over the last years some viruses have been detected as the cause of diarrhoea. Among the viral agents associated with these illnesses, Rotavirus is the most important so that their detection is epidemiologically important.

Rotavirus infections of adults are usually subclinical but occasionally cause illness in parents of children with Rotavirus diarrhoea, immunocompromised patients (including those with HIV), the elderly, and travelers to developing countries. In temperate climates, Rotavirus diarrhoea occurs predominantly during the fall and winter; in tropical setting and in developing countries, seasonality is less marked.

PRINCIPLE

Vitassay Rotavirus is a qualitative immunochromatographic assay for the detection of Rotavirus in human stool samples.

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

During the process, the sample reacts with the antibodies against Rotavirus, 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 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 Rotavirus**. 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.
- 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.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Rotavirus** ▪ 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

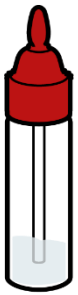
Collet sufficient quantity of faeces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

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

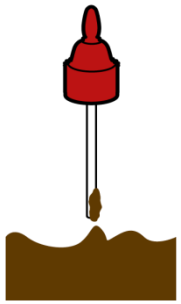
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, take 125µL of the sample using a micropipette and transfer it into vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

1



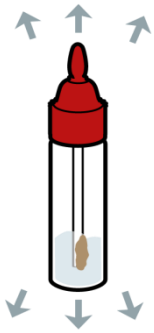
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

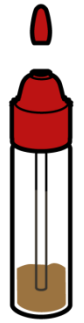
PROCEDURE

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 Rotavirus** 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.

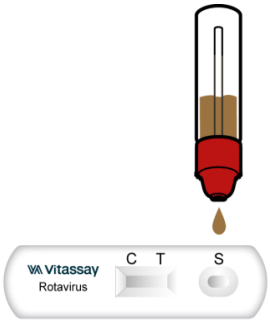
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

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 Rotavirus presence. No apparent infection caused by Rotavirus.
	**POSITIVE** In addition to the **green** line (**control line C**), a red line appears (**test line T**).	There is Rotavirus presence. Possible viral infection caused by Rotavirus.
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 Rotavirus**. 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.
- After one week of infection, the number of viruses in faeces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.
- The quality of **Vitassay Rotavirus** depends on the quality of the sample. Proper faecal specimens must be obtained.
- Positive results determine the presence of Rotavirus in faecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods or PCR) to confirm the results.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigens in the faecal sample is lower than the detection limit value. If symptoms or situation still persist, a Rotavirus determination should be carried out with another technique (for example PCR).
- **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

Diarrheic illnesses are one of the most common causes of morbidity and mortality between infants in developing countries, causing three million of deaths each year. Group A of human Rotavirus is the most frequent cause of acute diarrhoea.

Mortality due to Rotavirus infection in developing countries is greater than in developed countries; the frequency of the infection in developing and developed countries is similar.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation was performed comparing **Vitassay Rotavirus** and another commercial test (Ridascreen®*Rotavirus* ELISA Test) with faecal samples.

Results were as follows:

		Ridascreen®*Rotavirus* ELISA Test		
		Positive	Negative	Total
Vitassay Rotavirus	Positive	18	1	19
	Negative	0	43	43
	Total	18	44	62

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

Vitassay Rotavirus vs Ridascreen®*Rotavirus* ELISA Test			
Sensitivity	Specificity	PPV	NPV
>99%	98%	>94%	>99%

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

Subsequently**,** 33 samples from patients suspicious of Rotavirus infection were simultaneously evaluated with two rapid test, **Vitassay Rotavirus,** and a commercial kit:

		Stick Rotavirus		
		Positive	Negative	Total
Vitassay Rotavirus	Positive	22	0	22
	Negative	1	10	11
	Total	23	10	33

Table 3. Results of **Vitassay Rotavirus** compared to a commercial kit.

Vitassay Rotavirus vs Stick Rotavirus			
Sensitivity	Specificity	PPV	NPV
>95%	99%	>99%	>90%

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus** compared to a commercial kit.

In addition, another evaluation was performed with remnants of stool samples from 247 patients comparing Vitassay Adenovirus with a commercial immunochromatographic kit for the detection of Rotavirus and Adenovirus. Discrepant results were confirmed by PCR.

Results are shown below:

		Reference method		
		Positive	Negative	Total
Vitassay Rotavirus	Positive	97	1	98
	Negative	2	147	149
	Total	99	148	247

Table 5. Results of **Vitassay Rotavirus** compared to a commercial kit and confirmed by PC.

Reference method			
Sensibilidad	Especificidad	VPP	VPN
98.0% (92.9-99.8%)	99.3% (96.3 – 100%)	99.0% (94.4–100%)	98.7% (95.2 – 99.8%)

Table 6. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus** compared to a commercial kit and confirmed by PCR.

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

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Cross reactivity

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

Adenovirus	*Escherichia coli O157:H7*	*Salmonella typhi*
Astrovirus	*Giardia lambia*	*Shigella boydii*
Campylobacter coli	*Helicobacter pylori*	*Shigella dysenteriae*
Campylobacter jejuni	*Listeria monocytogenes*	*Shigella flexneri*
Clostridium difficile	Norovirus	*Shigella sonrei*
Cryptosporidium parvum	*Salmonella enteritidis*	*Staphylococcus aureus*
Entamoeba hystolitica	*Salmonella paratyphi*	*Yersinia enterocolitica*
Enterovirus	*Salmonella typhimurium*	

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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-7355008 Ed00 May 2016	Original version	05/2016
IUE-7355008 Ed01 October 2023	Addition of a new evaluation. 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.	03/10/2023

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