VITASSAY

Rotavirus+Norovirus

Rapid test for the simultaneous qualitative detection of rotavirus and norovirus genogroups I and II (GI and GII) in human stool samples.

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Vitassay

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For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Rotavirus+Norovirus is a rapid, immunochromatographic, one step assay for the simultaneous qualitative detection of rotavirus and norovirus genogroups I and II (GI and GII) in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of rotavirus and/or norovirus infection.

INTRODUCTION

Acute gastroenteritis is one of the major causes of morbidity and mortality in children worldwide. It is an infection of the gastrointestinal tract caused by a wide range of enteric pathogens, including bacteria, viruses, and parasites.

Cumulative data from previous studies shows that enteric viruses have replaced bacteria as the most significant pathogen of acute diarrhoea. Of these viruses, rotavirus and noroviruses have been recognized as the most common etiological agents of pediatric acute gastroenteritis.

Rotavirus is a double stranded RNA virus and belongs to the family of Reoviridae that includes seven serogroups (A-G). Group A rotavirus predominantly results in severe acute diarrhoea in children.

Norovirus classifieds into the family of *Caliciviridae* has a single-strand, positive sense, polyadenylated RNA genome that contains three open reading frames (ORFs). Norovirus have been recognized as another most common causative agent causing acute gastroenteritis in children.

Norovirus-associated acute gastroenteritis is characterized by the sudden onset of intense vomiting and dehydrating diarrhoea, typically lasting 1 to 3 days, with high rates of transmission to persons of all ages.

PRINCIPLE

Vitassay Rotavirus+Norovirus is a qualitative immunochromatographic assay for the detection of rotavirus and norovirus (GI and GII) in human stool samples**.**

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

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against norovirus (GI and GII).

During the process, the sample reacts with the antibodies against rotavirus (strip A) and/or norovirus (GI and GII) (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is rotavirus 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 norovirus 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.
- 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 Rotavirus + Norovirus**. Do not use any other commercial kit component or components from 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.

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- 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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Rotavirus+Norovirus** ▪ 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 1-2mL 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.

Homogenise the stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

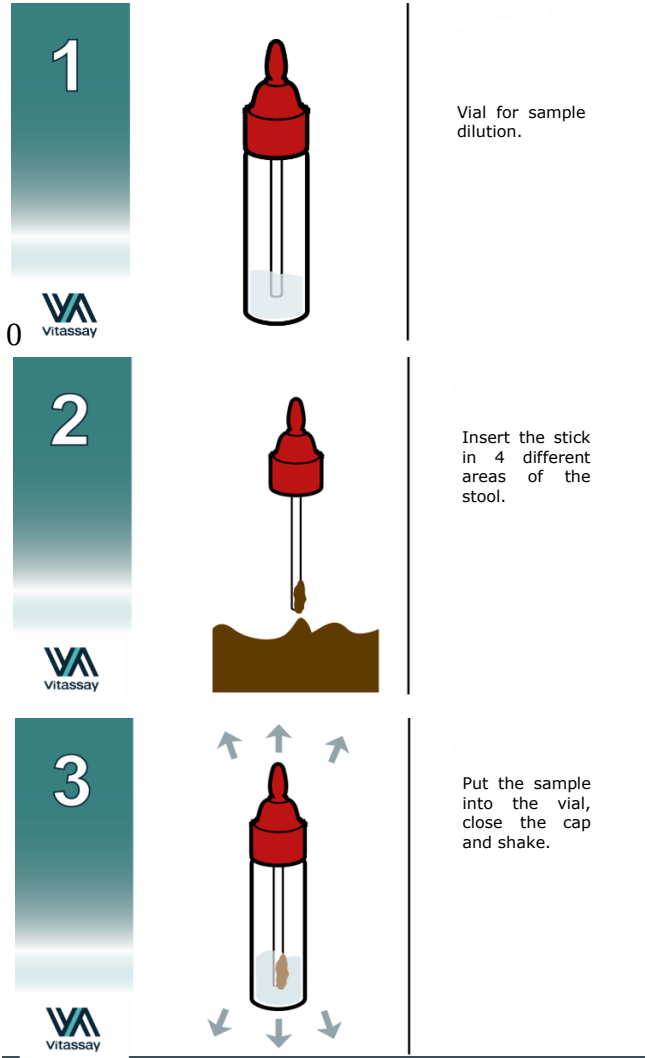
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for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

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 vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Rotavirus+Norovirus** 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 – rotavirus (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter B – norovirus (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.



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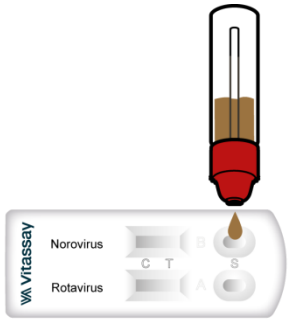
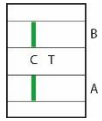
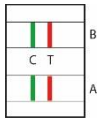
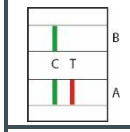
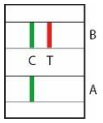


Dispense 4 drops in the circular window marked with the letter S to the strip B – Norovirus

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Rotavirus	Strip B Norovirus	INTERPRETATION
	**Negative**	**Negative**	There is no rotavirus and/or norovirus (GI/ GII) presence. There is no aparent infection caused by rotavirus and/or norovirus (GI/GII).
	GREEN	GREEN	
	**Positive**	**Positive**	There is rotavirus and norovirus (GI/GII) presence. Possible infection caused by rotavirus and norovirus.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is rotavirus presence. Possible nfection caused by rotavirus.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is norovirus (GI and/or GII) presence. Possible infection caused by norovirus.
	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 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 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 Rotavirus+Norovirus**. 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 presence of virus shed in stool decreases considerably so a lower concentration in the stool sample is probable. The stool sample should be taken within the first week of symptom onset.
- The quality of **Vitassay Rotavirus + Norovirus** depends on the quality of the sample. Proper faecal specimens must be obtained.
- Positive results determine the presence of adenovirus and/or rotavirus in faecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods or PCR) to confirm the results. 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 in the faecal sample is lower than the detection limit value. If symptoms or situation still persist, a rotavirus and/or norovirus 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

An estimated four billion cases of diarrhoea and over one million diarrhoea-related deaths occur worldwide annually.

Viruses, specifically Rotavirus A (RoV) and genogroup I and II Norovirus (GI and GII) are predominant causes of viral gastroenteritis worldwide, and are responsible for over 40% of all cases of diarrhoea in developing countries.

Based on previous studies, rotavirus infections caused 25 million clinical visits, 2 million hospital admissions and about 611000 deaths annually worldwide in children, mainly in developing countries.

Norovirus is a leading cause of diarrhoeal disease among older children and adults, and the leading cause of diarrhoeal disease outbreaks worldwide.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with faecal samples was performed comparing **Vitassay Rotavirus+Norovirus** and other two commercial tests (Ridascreen®*Rotavirus* ELISA Test, r-Biopharm) for rotavirus and (Simple Norovirus, Operon) for norovirus GI/GII and confirmed by PCR.

Results were as follows:

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

Table 1. Results of **Vitassay Rotavirus+Norovirus** (Rotavirus) compared to a commercial ELISA kit.

Vtassay Rotavirus+Norovirus (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+Norovirus** (Rotavirus) compared to a commercial ELISA kit**.**

		Simple Norovirus		
		Positive	**Negative**	**Total**
Vitassay Rotavirus +Norovirus	**Positive**	2	0	2
	Negative	0	48	48
Norovirus GI	**Total**	2	48	50

Table 3. Results of **Vitassay Rotavirus+Norovirus** (norovirus GI) compared to a commercial immunochromatographic kit.

Vitassay Rotavirus + Norovirus (Norovirus GI) **vs Simple Norovirus**

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Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Norovirus** (Norovirus GI) compared to a commercial immunochromatographic kit.

		PCR		
		Positive	Negative	Total
Vitassay Rotavirus + Norovirus Norovirus GI	Positive	2	0	2
	Negative	0	48	48
	Total	2	48	50

Table 5. Results of **Vitassay Rotavirus+Norovirus** (Norovirus GI) confirmed with a PCR.

Vitassay Rotavirus+Norovirus (Norovirus GI) **vs PCR**			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Table 6. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Norovirus** (Norovirus GI) confirmed by PCR.

		Simple Norovirus		
		Positive	Negative	Total
Vitassay Rotavirus +Norovirus norovirus GII	Positive	10	0	10
	Negative	0	48	48
	Total	10	48	58

Table 7. Results of **Vitassay Rotavirus+Norovirus** (norovirus GII) compared to a commercial immunochromatographic kit.

Vitassay Rotavirus+Norovirus (Norovirus GII) **vs Simple Norovirus**			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Table 8. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Norovirus** (Norovirus GII) compared to a commercial immunochromatographic kit**.**

		PCR		
		Positive	Negative	Total
Vitassay Rotavirus + Norovirus norovirus GII	Positive	8	0	8
	Negative	2	48	50
	Total	10	48	58

Table 9. Results of **Vitassay Rotavirus+Norovirus** (Norovirus GII) confirmed by PCR.

Vitassay Rotavirus + Norovirus (Norovirus GII) **vs PCR**			
Sensitivity	Specificity	PPV	NPV
80%	>99%	>99%	96%

Table 10. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Norovirus** (Norovirus GII) confirmed by PCR. Results showed that **Vitassay Rotavirus+Norovirus** has high sensitivity and specificity to detect rotavirus and norovirus (GI and/or GII).

Cross reactivity

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

Adenovirus	*Escherichia coli O157:H7*	*Salmonella typhimurium*
Astrovirus	*Giardia lamblia*	*Salmonella typhi*
Campylobacter coli	Hepatitis A virus	*Shigella boydii*
Campilobacter jejuni	*Helicobacter pylori*	*Shigella dysenteriae*
Clostridium difficile	*Listeria monocytogenes*	*Shigella flexneri*
Cryptosporidium parvum	Norovirus (Strip A)	*Shigella sonnei*
Enterovirus	Rotavirus (Strip B)	*Staphylococcus aureus*
Entamoeba hystolitica (Strip A)	*Salmonella enteritidis*	*Yersinia enterocolitica*
Escherichia coli O111 (Strip B)	*Salmonella paratyphi*	RSV (Strip B)

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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-7455015 Ed02 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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