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

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 diarrhea. 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 diarrhea 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 diarrhea, 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.
- 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. Device for single use.
- 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 Rotavirus+Norovirus**. 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.

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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.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

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

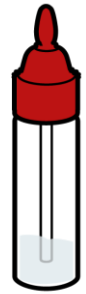
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

Homogenize stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

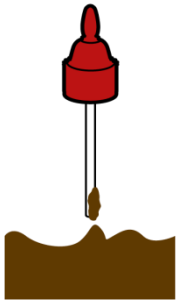
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for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

1



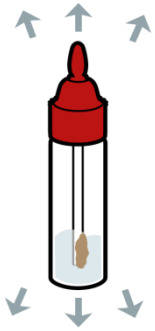
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

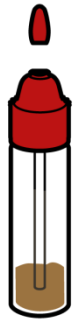
PROCEDURE

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 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.

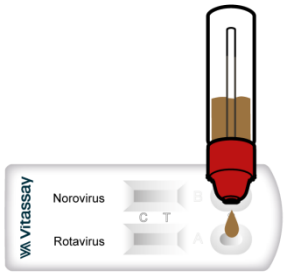
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



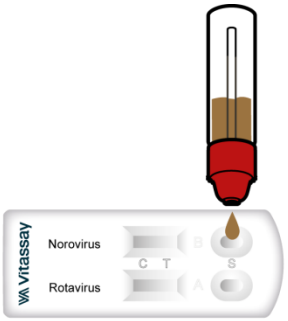
Cut the end of the cap.

5



Dispense 4 drops in the circular window marked with the letter S to the strip A – Rotavirus.

6



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

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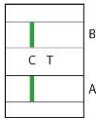
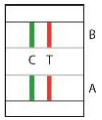
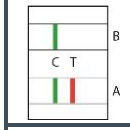
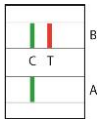
INTERPRETATION OF THE RESULTS

RESULTS	Strip A Rotavirus	Strip B Norovirus	INTERPRETATION
	**Negative**	**Negative**	There is no rotavirus and/or norovirus (GI and GII) presence. There is not infection caused by rotavirus and/or norovirus (GI and GII).
	GREEN	GREEN	
	**Positive**	**Positive**	There is rotavirus and norovirus (GI and/or GII) presence. Infection caused by rotavirus and norovirus.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is rotavirus presence. Infection caused by rotavirus.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is norovirus (GI and/or GII) presence. 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 or deterioration of the reagents are the main reasons of 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 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

- **Vitassay Rotavirus+Norovirus** 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 week of infection, the number of viruses in feces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay Rotavirus+Norovirus** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of rotavirus and or norovirus (GI and/or GII) in fecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods, PCR or microscopy) 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 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 microscopy or PCR).

EXPECTED VALUES

An estimated four billion cases of diarrhea and over one million diarrhea-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 diarrhea 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 diarrheal disease among older children and adults, and the leading cause of diarrheal disease outbreaks worldwide.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with fecal 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 rotavirus	Positive	18	1	19
	Negative	0	43	43
	Total	18	44	62

Vitassay Rotavirus+Norovirus (rotavirus) **vs Ridascreen®*Rotavirus* ELISA Test**			
Sensitivity	Specificity	PPV	NPV
>99%	98%	>94%	>99%

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

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

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

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

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

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

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

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

Results showed that **Vitassay Rotavirus+Norovirus** has a 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 feces:

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

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