VITASSAY

Norovirus

Rapid test for the qualitative detection of 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 Norovirus is a rapid one step immunochromatographic assay for the qualitative detection of Norovirus genogroups I and II (GI and GII) in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of Norovirus (GI and GII) infection.

NTRODUCTION

Noroviruses are a group of no enveloped single-stranded positive-sense RNA viruses classified in the family *Caliciviridae*.

Norovirus can infect humans via multiple routes, including the oral route, transmitted through contact with faecal matter or aerosolized vomitus from infected people, as well as contaminated surfaces, food, or water.

After incubation period 12 to 48h, Norovirus illness is characterized by projectile vomiting, no bloody diarrhoea, nausea, abdominal cramps, and low-grade fever. Some persons might experience only vomiting or diarrhoea. In healthy individuals, the duration of symptoms is usually not longer than 48 h, and the disease is self-limiting in most patients. However, young children and the elderly are at increased risk for more severe and prolonged illness leading hospitalization, while the disease is increasingly recognized as an important cause of chronic gastroenteritis for immunocompromised patients.

Although Norovirus can be detected in rectal swabs and vomitus, whole-stool samples are the preferred clinical specimen for the detection of Norovirus because they contain a higher quantity of virus.

PRINCIPLE

Vitassay Norovirus is a qualitative immunochromatographic assay for the detection of Norovirus genogroups I and II (GI and GII) in human stool samples.

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

During the process, the sample reacts with the antibodies against Norovirus (GI and GII), 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 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 Norovirus**. 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).
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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 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)

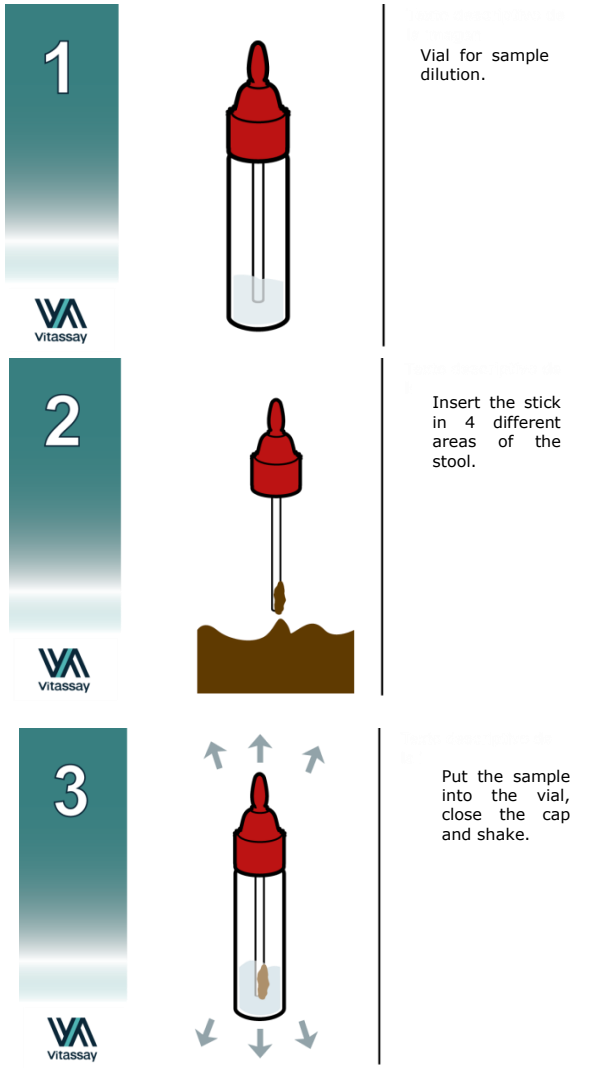
SPECIMEN COLLECTION

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 the 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).

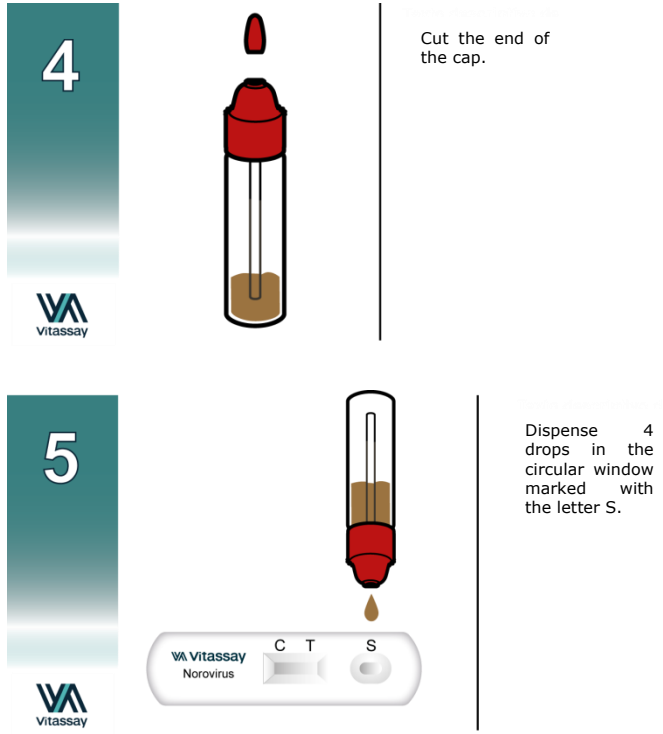


PROCEDURE

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 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 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.



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INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one **green** line in the control zone **(C)**.	There is not presence of Norovirus No apparent infection caused by Norovirus (GI and/or GII).
	**POSITIVE** In addition to the **green** line (**control line C**), a **red** line appears (**test line T**).	There is presence of Norovirus. Possible infection caused by Norovirus (GI and/or GII).
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 control is included in **Vitassay Norovirus**. 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 Norovirus** depends on the quality of the sample. Proper faecal specimens must be obtained.
- 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.
- 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 Norovirus determination should be carried out with another technique (for example PCR).
- **Bloody stool** samples and/or **mucinous/presence of mucus** 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

Noroviruses are the leading cause of epidemic and sporadic cases of acute gastroenteritis worldwide and a leading cause of foodborne disease.

The majority of Norovirus outbreaks occur in health care settings (including long-termcare facilities and hospitals), where the virus is predominantly spread from person to person. In addition, Noroviruses have also been identified in over 58% of the reported foodborne outbreaks in which an etiologic agent was determined. In the most recent disease burden estimates in the United Satetes (US), Noroviruses causes 570 to 800 deaths, 56,000 to 71,000 hospitalizations, 400,000 emergency room visits, and 1.7 to 1.9 million outpatient visits anually. In pediatric populations in industrialized countries where a rotavirus vaccine has been introduced, Norovirus are rapidly replacing rotavirus as the most common cause of medically attended acute gastroenteritis.

In countries that belong to temperate latitudes, most infections occur in the fall and winter and at least 70% of outbreaks are reported in semiclosed communities such as long-term-care facilities, schools, hospitals, and cruise ships.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with faecal samples was performed using **Vitassay Norovirus** and these results were compared with a commercially available immunochromatographic test (Simple Norovirus, Operon) and confirmed by PCR.

Results were as follows:

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

Table 1. Results of **Vitassay Norovirus** (Norovirus GI) compared to a commercial immunochromatographic kit.

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

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

		PCR		
		Positive	**Negative**	**Total**
Vitassay Norovirus Norovirus GI	**Positive**	2	0	2
	Negative	0	48	48
	Total	2	48	50

Table 3. Results of **Vitassay Norovirus** (Norovirus GI) vs PCR

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

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus** (Norovirus GI) vs PCR.

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

Table 5. Results of **Vitassay Norovirus** (Norovirus GII) **compared** to a commercial immunochromatographic kit.

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

Table 6. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus** (Norovirus GII) **compared** to a commercial immunochromatographic kit.

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

Table 7. Results of **Vitassay Norovirus** (Norovirus GII) **vs** PCR.

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

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Table 8. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus** (Norovirus GII) **vs** PCR.

A second clinical study was carried out in which 174 faecal sample remnants from patients with suspected norovirus infection were analyzed. In this case, a commercial immunochromatographic kit was also used as a reference method. Discrepant results were confirmed by PCR.

The results are shown below, after analysis of the discrepancies:

		Reference method		
		Positivo	Negativo	Total
Vitassay Norovirus	Positivo	56	1	57
	Negativo	3	114	117
	Total	59	115	174

Table 9. Results of **Vitassay Norovirus** compared to the reference method.

Vitassay Norovirus VS Reference method			
Sensitivity	Specificity	PPV	NPV
94.9% (85.9-98.9%)	99.1% (95.3–100%)	98.2% (90.6-100%)	97.4% (92.7-99.5%)

Table 10. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus** compared to the reference.

The results showed that **Vitassay Norovirus** has a high sensitivity and specificity to detect Norovirus (GI and GII).

Analytical sensitivity

Limit of Detection of **Vitassay Norovirus** has been established on 12.5ng/mL for Norovirus GI and 5ng/mL for Norovirus GII.

Cross-reactivity

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

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

REFERENCES

1. JAN VLNJÉ. "Advances in Laboratory Methods for Detection and Typing of Norovirus". Journal of Clinical Microbiology 53:373-381. 2015.

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-7355013 Ed03 October 2023	Addition of a new evaluation. The format has been updated. Limitations section has been updated. Analytical sensitivity section has been added. Correction of transcription error in Interpretation of results section. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability. Material required but not included updated with minor changes. The wording has been changed under Interpretation of results.	03/10/2023

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