VITASSAY

Norovirus GI+GII

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

IUE-7455014 Ed02 October 2023

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Vitassay

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For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Norovirus GI+GII is a rapid, immunochromatographic, one step assay for the simultaneous qualitative detection of Norovirus GI and Norovirus GII in human stool samples.

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

INTRODUCTION

Norovirus is a genus within the *Caliciviridae* family, classified into at least 6 genogroups (GI-GVI) and further into 29 genotypes based on the major viral capsid protein (VP1), including the 3 genogroups that infect humans (GI, GII, and GIV).

In spite of this massive genetic heterogeneity, the vast majority of human Norovirus infections are associated with GII viruses.

Norovirus is a clinically important RNA virus that is estimated to cause almost half of all cases of acute gastroenteritis, globally. Norovirus is also the only human enteric virus known to cause pandemics of acute gastroenteritis. Norovirus infects people of all ages; however, the majority of Norovirus-associated gastroenteritis outbreaks occur within institutional settings, such as aged-care facilities, hospitals and childcare centers, thereby affecting the most vulnerable in the community including the elderly, immunocompromised and young children.

Noroviruses 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 an incubation period of 12 to 48 h, norovirus illness is characterized by projectile vomiting, non-bloody diarrhea, nausea, abdominal cramps, and low-grade fever. Some persons might experience only vomiting or diarrhea. 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 to hospitalization, while the disease is increasingly recognized as an important cause or chronic gastroenteritis in immunocompromised patients.

PRINCIPLE

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

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

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

During the process, the sample reacts with the antibodies against Norovirus GI (strip A) and/or Norovirus GII (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is Norovirus GI 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 GII 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 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 GI + GII** 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,

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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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Norovirus GI+GII** ▪ 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

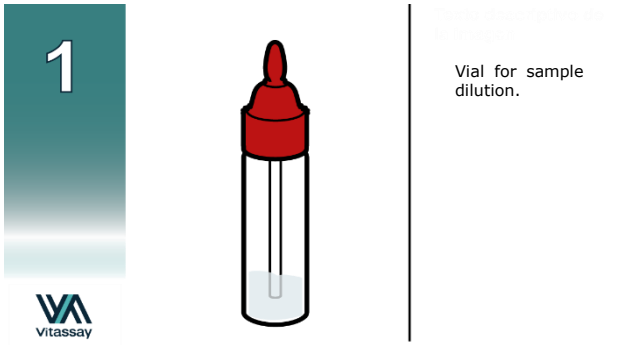
Collect sufficient quantity of faeces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

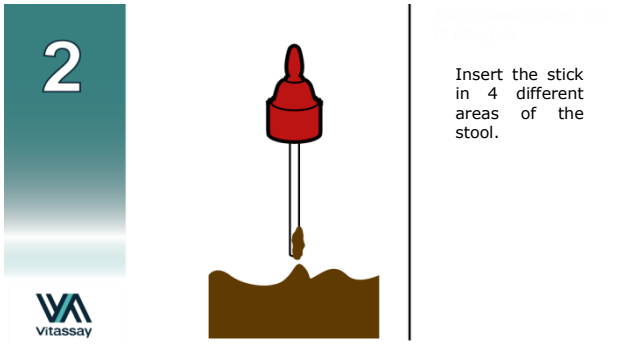
Samples can be stored in the refrigerator (2-8ºC) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC. Samples must be brought to room temperature before testing.

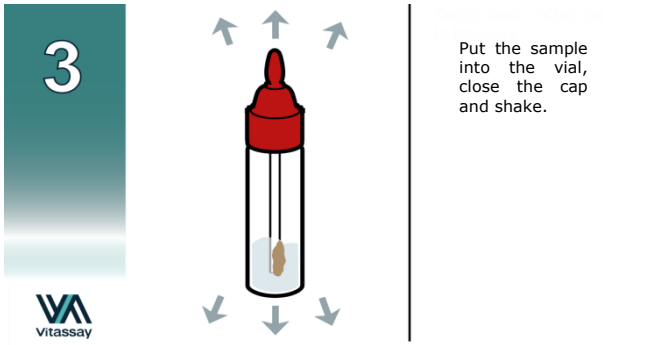
Homogenise stool samples as thoroughly as possible prior to preparation.

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).





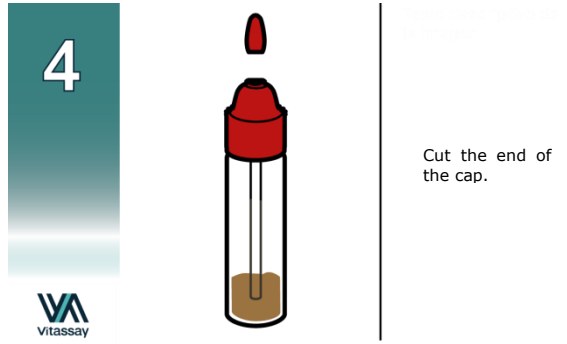


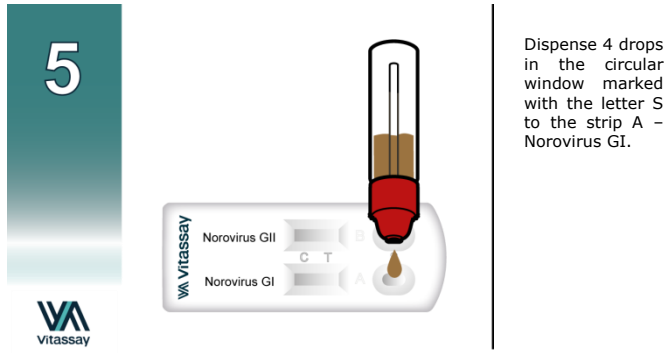
PROCEDURE

Allow the test, stool sample, controls (if applicable) 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 Norovirus GI+GII** 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 for the strip A – Norovirus GI (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter S for the strip B – Norovirus GII (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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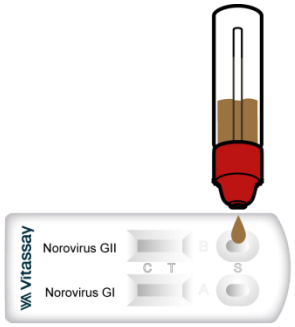
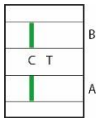
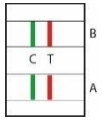
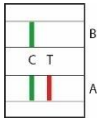
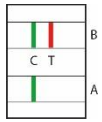


Dispense 4 drops in the circular window marked with the letter S to the strip B – Norovirus GII.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Norovirus GI	Strip B Norovirus GII	INTERPRETATION
	**Negative**	**Negative**	There is no Norovirus GI or Norovirus GII presence. No apparent infection caused by Norovirus GI or Norovirus GII.
	GREEN	GREEN	
	**Positive**	**Positive**	There is Norovirus GI and Norovirus GII presence. Possible infection caused by Norovirus GI and Norovirus GII.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is Norovirus GI presence. Possible infection caused by Norovirus GI.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is Norovirus GII presence. Possible nfection caused by norovirus GII.
	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-coloured 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 Norovirus GI+GII**. Green lines appearing in the 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 Norovirus GI+GII** 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. 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 adenovirus 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

The majority of norovirus outbreaks occur in health care settings (including long-term care facilities and hospitals), where the virus is predominantly spread from person to person. In addition, norovirus 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 States, norovirus causes 570 to 800 deaths, 56000 to 71000 hospitalizations, 400000 emergency room visits, and 1.7 to 1.9 million outpatient visits annually.

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

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation was performed using **Vitassay Norovirus GI+GII** and these results were compared with a commercial immunochromatographic test (Simple Norovirus, Operon). Results were later confirmed by a PCR.

Results were as follows:

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

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

Vitassay Norovirus GI+GII (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 GI+GII** (Norovirus GI) compared to a commercial immunochromatographic kit.

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

Table 3. Results of **Vitassay Norovirus GI+GII** (Norovirus GI) confirmed with PCR.

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Vitassay Norovirus GI+GII (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 GI+GII** (Norovirus GI) confirmed with PCR.

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

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

Vitassay Norovirus GI+GII (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 GI+GII** (Norovirus GII) compared to a commercial immunochromatographic kit.

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

Table 7. Results of **Vitassay Norovirus GI+GII** (Norovirus GII) confirmed with PCR.

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

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

In addition, **Vitassay Norovirus GI+GII** was evaluated with 174 remants of stool samples from patients suspected of gastrointestinal infection compared with a commercial immunochromatographic kit and subsequently a PCR to confirm the presence of **Norovirus GI+GII** in the discrepant samples. Strip A and B were tested separately:

Results strip A (NV GI) after assessing the discrepant results:

		Reference method		
		Positive	**Negative**	**Total**
Vitassay Norovirus GI+GII (NV GI)	**Positive**	4	0	4
	Negative	1	169	170
	Total	5	169	174

Table 9. Results of **Vitassay Norovirus GI+GII** (strip A; Norovirus GI) vs the reference method.

Reference method			
Sensitivity	**Specificity**	**PPV**	**NPV**
80.0% (28.4-99.5)	100% (97.8–100)	100% (39.8–100)	94.4% (96.8–100)

Table 10. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus GI+GII** (strip A; Norovirus GI) vs the reference method.

Results strip B (NV GII) after assessing the discrepant results:

		Reference method		
		Positive	**Negative**	**Total**
Vitassay Norovirus GI+GII (NV GI)	**Positive**	52	1	53
	Negative	2	119	121
	Total	54	120	174

Table 11. Results of **Vitassay Norovirus GI+GII** (strip B; Norovirus GII) vs the reference method.

Reference method			
Sensitivity	**Specificity**	**PPV**	**NPV**
96.3% (87.3-99.5)	99.2% (95.4-100)	98.1% (89.9–100)	98.3% (94.2 –99.8)

Table 12. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Norovirus GI+GII** (strip B; Norovirus GII) vs the reference method.

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

Cross reactivity

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

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

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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		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
CE	CE Marking		

Changes control		
Nº Version	**changes**	**Date**
IUE-7455014 Ed02 October 2023	Addition of new clinical 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. The wording has been changed under Interpretation of results.	03/10/2023

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