todylaboratories®

Rapid Tests

CE IVD REF C61RT1021

Norovirus Rapid Test Cassette (Feces) Package Insert

A rapid, one step test for the qualitative detection of Norovirus in human feces.
For professional in vitro diagnostic use only.

INTENDED USE

The Norovirus Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of Norovirus in human fecal specimens to aid in the diagnosis of Norovirus infection.

SUMMARY

Noroviruses (NoV) are a genetically diverse group of single stranded RNA, nonenvelopped viruses belonging to the Caliciviridae family. For decades they were called "small round structured viruses" (SRSV) or "Norwalk-like viruses" until recently when their taxonomy was investigated using modern molecular techniques. Initially four antigenic types of SRSV were recognized, but more recently three genogroups have been identified with the genus Norovirus. Genogroup 1 and Genogroup 2 are associated with human infections whilst Genogroup 3 is associated with bovine and porcine infection.

Noroviruses are a major cause of acute gastroenteritis worldwide, often causing explosive outbreaks in institutions. They are highly contagious, with an inoculum of as few as ten particles being able to cause infection. Transmission occurs through ingesting contaminated food and water and by person-to-person spread. Transmission is predominantly fecal-oral but may be airborne due to aerosolisation of vomitus, which typically contains abundant infectious virus particles. Outbreaks may involve several routes of transmission. The illness is acute, usually mild, although it has caused fatalities among the frail elderly, and self-limiting and follows an incubation period of 24-48 hours although cases can occur within 12 hours of exposure. The ability of Noroviruses to cause outbreaks in institutions has become a major public health issue. Outbreaks of Norovirus infection can be associated with restaurants and institutions as diverse as nursing homes, hospitals and elite sporting camps. Infections in infants, elderly or frail patients can be fatal if left untreated.

The symptoms of Norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. In most people the illness is self-limiting with symptoms lasting for about 1 or 2 days. In general, children experience more vomiting than adults.

PRINCIPLE

The *Norovirus* Test Cassette (Feces) is a qualitative, lateral flow immunoassay for the detection of *Norovirus* in human fecal specimens.

The assay uses Genogroup 1 and Genogroup 2 specific monoclonal antibodies coated on the test membrane. During testing, the stool specimen reacts with the conjugate antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with Genogroup 1 and 2 antibodies on the membrane and generates a colored line at the level of the T1 and T2 region respectively. The presence of a colored line in T1 region indicates a positive result for Genogroup 1 and in T2 region for Genogroup 2 respectively, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control reaction region (C) indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains Genogroup 1 and Genogroup 2 monoclonal antibody coated particles and Genogroup 1 and Genogroup 2 monoclonal antibodies coated on the membrane.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- The test cassette should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use test if pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch containing desiccant until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- Viral detection is improved by collecting the specimens at the onset of the symptoms. It has been reported that the maximum excretion of Norovirus in the feces of patients with gastroenteritis occurs 3-13 days after onset of symptoms. If the specimens are collected long after the onset of diarrheic symptoms, the quantity of antigen may not be sufficient to obtain a positive reaction or the antigens detected may not be linked to the diarrheic episode.
- The fecal specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
- Bring the necessary reagents to room temperature before use.

MATERIALS

Materials provided

• Test Cassettes • Package Insert • Specimen collection tube with extraction buffer

Materials required but not provided

• Specimen Collection Containers • Timer • Droppers • Centrifuge and pipette to dispense 80 µL if required

DIRECTIONS FOR USE

Allow the test, specimen, buffer, and/or controls to reach room temperature (15-30°C) prior to testing.

1. To collect fecal specimens:

Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long term storage, specimens should be kept below -20°C.

2. To process fecal specimens:

• For **Solid Specimens**: Unscrew the cap of the specimen collection tube, then randomly **stab the specimen collection applicator into the fecal specimen in at least 3 different sites** to collect approximately **50 mg of feces** (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.

• For **Liquid Specimens**: Hold the dropper vertically, aspirate fecal specimens, and then transfer **50 µl** into the specimen collection tube containing the extraction buffer.

Tighten the cap onto the specimen collection tube, then **shake the specimen collection tube vigorously** to mix the specimen and the extraction buffer.

3. Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.

4. Hold the specimen collection tube upright and **unscrew the small cap** of the specimen collection tube. Invert the specimen collection tube and **transfer 2 full drops of the extracted specimen** (approximately 80 µL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.

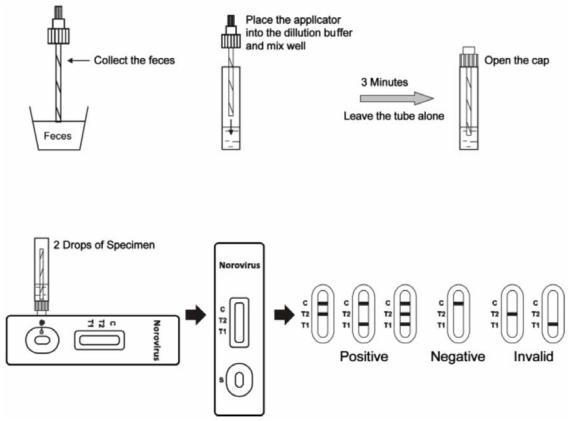
5. **Read the results at 15 minutes after dispensing the specimen.** Do not read results after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the diluted sample contained in the extraction buffer vial. Collect 80 µL of supernatant, dispense into the specimen well (S). Start the timer and continue from step 5 onwards in the above instructions for use.

INTERPRETATION OF RESULTS

(Please refer to the illustration above)

Genogroup 1 POSITIVE:* Two distinct colored lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the Genogroup 1 region (T1).



Genogroup 2 POSITIVE:* Two distinct colored lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the Genogroup 2 region (T2).

Genogroup 1 & Genogroup 2 POSITIVE:* Three distinct colored lines appear. One colored line should be in the control region (C) and two colored line should be in the Genogroup 1 region (T1) and Genogroup 2 region (T2). A positive result in the Genogroup 1 region and Genogroup 2 region indicates that Genogroup 1 antigen and Genogroup 2 antigen were detected in the sample.

***NOTE**: The intensity of the color in the test line region (T) will vary depending on the concentration of Norovirus antigen present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.

NEGATIVE: One colored line appears in the control line region (C). No line appears in the test line regions (T1/T2).

INVALID: Control line (C) fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. This test should be used for detection of Norovirus antigens in human stool only.
2. The Norovirus Rapid Test Cassette only indicates the presence of Norovirus antigen in the specimen and should not be used as the sole criteria for the diagnosis of Norovirus infection.
3. Stool sample from infant under one year old can produce a false positive result.
4. As with all diagnostic tests, result must be considered together with other clinical information available to the physician.
5. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Norovirus infection.

EXPECTED VALUES

The Norovirus Rapid Test Cassette (Feces) has been compared with RT-PCR method, demonstrating an overall accuracy of 94.3%.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

The performance of the Norovirus Rapid Test Cassette has been evaluated with 70 clinical specimens collected from children and young adults in comparison with RT-PCR method. The results show that the relative sensitivity of the Norovirus Rapid Test Cassette (Feces) is 95.7% and the relative specificity is 91.7%.

One Step Norovirus Rapid Test Cassette vs. RT-PCR

Method		RT-PCR		Total Results
One Step Norovirus Rapid Test Cassette	Results	Positive	Negative	
	Positive	44	2	46
	Negative	2	22	24
Total Results		46	24	70

Relative Sensitivity: 95.7% (95%CI:*85.16%-99.57%)
Relative Specificity: 91.7% (95%CI:*73.00%-98.97%)
Relative Accuracy: 94.3% (95%CI:*86.01%-98.42%) *Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of four specimens: a negative, a low positive, a medium positive and a high positive. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same four specimens: a negative, a low positive, a medium positive and a high positive. The specimens were correctly identified >99% of the time.

Cross-reactivity

Cross reactivity with following organisms has been studied at 1x10^{7}organisms/ml. The following organisms were found negative when tested with the Norovirus Rapid Test Cassette (Feces).

Corynebacterium diphtheria	*Pseudomonas aeruginosa*	*Enterococcus faecalis*
Shigella dysenteriae	*Candida albicans*	*Neisseria gonorrhea*
Shigella flexneri	*Proteus vulgaris*	*Enterococcus faecium*
Proteus mirabilis	*Shigella sonnei*	*Clostridium difficile*
Gardnerella vaginalis	*Helicobacter pylori*	*E.coli*

BIBLIOGRAPHY

1. Shiota, T., Okame, M., Takanashi, S., Khamrin, P., Takagi, M., Satou, K., Masuoka, Y., Yagyu, F., Shimizu, Y., Kohno, H., Mizuguchi, M., Okitsu, S., Ushijima, H. (2007). Characterization of a Broadly Reactive Monoclonal Antibody against Norovirus Genogroups I and II: Recognition of a Novel Conformational Epitope. J. Virol. 81: 12298- 12306
2. Nguyen, T. A., Khamrin, P., Takanashi, S., Le Hoang, P., Pham, L. D., Hoang, K. T., Satou, K., Masuoka, Y., Okitsu, S., Ushijima, H. (2007). Evaluation of Immunochromatography Tests for Detection of Rotavirus and Norovirus among Vietnamese Children with Acute Gastroenteritis and the Emergence of a Novel Norovirus GII.4 Variant. J Trop Pediatr 53: 264-269
3. M Okame, T Shiota, G Hansman, M Takagi, F Yagyu, S Takanashi, TG Phan, Y Shimizu, H Kohno, S Okitsu, H Ushijima (2007). Anti-norovirus polyclonal antibody and its potential for development of an antigen-ELISA. J Med Virol (2007) 79: 1180-6.
4. Tracy Dewese Parker & al., Identification of genogroup I and genogroup II broadly reactive epitopes on the norovirus capsid, Journal of Virology, June 2005: 7402-7409.

todylaboratories®

Tody Laboratories Int. SRL
22 Vadul Moldovei, s1, OP 18, Bucharest, CP 014033, ROMANIA
+4021 269 00 00 +4021 269 06 41
ISO 15189, 17043, 9001, 13485
www.todylaboratories.com