PathFlow™ Norovirus (EN)

MICROGEN BIOPRODUCTS

Instructions for Use

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

INTENDED USE

The PathFlow™ Norovirus Cassette is a rapid chromatographic immunoassay for the qualitative detection of Norovirus in human faeces specimens to aid in the diagnosis of Norovirus infection.

SUMMARY

Noroviruses (NoV) are a genetically diverse group of single stranded RNA, nonenveloped 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 faecal-oral but may be airborne due to aerosolization 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, diarrhoea, 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 PathFlow™ Norovirus Cassette is a qualitative, lateral flow immunoassay for the detection of Norovirus in human faeces 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 coloured line at the level of the T1 and T2 zone respectively. The presence of a coloured 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 coloured line will always appear in the control reaction zone (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.

MATERIALS

Provided: Test cassettes, Package Insert/Instructions for Use, and Specimen collection tube with extraction buffer.

Not Provided: Specimen collection / stool container, timer, Centrifuge and pipette to dispense 80 µL if required.

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use test if pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the 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 at room temperature or refrigerated (2°C-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 until use.
- **DO NOT FREEZE.**
- Do not use beyond the expiration date.

SPECIMENS

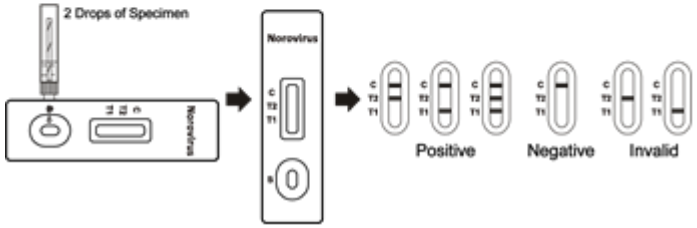
1. Viral detection is improved by collecting the specimens at the onset of the symptoms. It has been reported that the maximum excretion of Norovirus in the faeces of patients with gastroenteritis occurs 3-13 days after onset of symptoms. If the specimens are collected long after the onset of diarrheic 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.
2. The faeces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
3. Bring the necessary reagents to room temperature before use.

DIRECTIONS FOR USE / INSTRUCTIONS FOR USE

Allow the test, specimen, stool collection and buffer to equilibrate to room temperature (15-30°C) prior to testing.

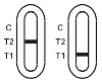
1. To collect faecal specimens:
   Collect sufficient quantity of faeces (1-2 ml or 1-2 g) in a clean, dry specimen collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2°C-8°C if not tested within 6 hours. For long-term storage, specimens should be kept below -20°C.
2. To process faecal specimens:
   • For Solid Specimens:
   Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the faecal specimen in at least 3 different sites to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). Do not scoop the faecal specimen.
   • For Liquid Specimens:
   Hold the dropper vertically, aspirate faecal specimens, and then transfer 2 drops of the liquid specimen (approximately 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 within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
4. Hold the specimen collection tube upright and open the cap on the tip. 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 extracted specimen 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

Positive:	
	**T1 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).
	**T2 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).
	**T1 & T2 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 region (T).
	**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.

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 is 95.7% and the relative specificity is 91.7%.

Method	Results	RT-PCR		Total Results
		Positive	Negative	
Norovirus Rapid Test Cassette	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

EXPECT VALUE

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

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 1.0 x 10^7 organisms/ml. The following organisms were found negative when tested with the Norovirus Rapid Test Cassette.

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

BIBLIOGRAPHY

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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.

TABLE OF SYMBOLS

SYMBOL	DEFINITION
LOT	Batch Code/Lot Number
IVD	In Vitro Diagnostic Medical Device
REF	Catalogue Reference
	Upper and Lower Temperature Limit
	Expiry Date
	Manufacturer and Address Details
	Date of Manufacture
	Please consult Instructions for Use
Σ	Sufficient for
	Do not use if package is damaged
CE	CE Mark
	Caution

This document applies to the following product codes:
M596CE / M596a / M596b

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