Norovirus/Rotavirus/Adenovirus Combo Rapid Test Cassette (Feces) Package Insert

REF INRA-635	English

A rapid, one step test for the qualitative detection of norovirus, rotavirus and adenovirus in human feces.

For professional in vitro diagnostic use only.

【INTENDED USE】

The Norovirus/Rotavirus/Adenovirus Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of norovirus, rotavirus and adenovirus in human feces specimens to aid in the diagnosis of norovirus, rotavirus or adenovirus infection.

【SUMMARY】

Norovirus Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of norovirus in human feces specimen. The test utilizes antibody specific for norovirus to selectively detect norovirus from human feces specimens.

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 faecal-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.

Rotavirus and Adenovirus Combo Test is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human feces specimen, providing results in 15 minutes. The test utilizes antibody specific for rotavirus and adenovirus to selectively detect rotavirus and adenovirus from human feces specimens. Acute diarrhea disease in young children is a major cause of morbidity worldwide and is a leading cause of mortality in developing countries.[1] Rotavirus is the most common agent responsible for acute gastroenteritis, mainly in young children.[2] Its discovery in 1973 and its association with infantile gastroenteritis represented a very important advancement in the study of gastroenteritis not caused by acute bacterial infection. Rotavirus is transmitted by oral-fecal route with an incubation period of 1-3 days. Although specimen collections taken within the second and fifth day of the illness are ideal for antigen detection, the rotavirus may still be found while diarrhea continues. Rotaviral gastroenteritis may result in mortality for populations at risk such as infants, the elderly and immunocompromised patients.[3] In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported.[4] With hospitalized children suffering from acute enteric disease up to 50% of the analyzed specimen were positive for rotavirus.[5] The viruses replicate in the cell nucleus and tend to be host species specific producing a characteristic cytopathic effect (CPE). Because rotavirus is extremely difficult to culture, it is unusual to use isolation of the virus in diagnosing an infection. Instead, a variety of techniques have been developed to detect rotavirus in feces.

Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhea in many of these children, second only to the rotaviruses.[6,7,8,9] These viral pathogens have been isolated throughout the world, and can cause diarrhea in children year round. Infections are most frequently seen in children less than two years of age, but have been found in patients of all ages. Further studies indicate that adenoviruses are associated with 4 - 15% of all hospitalized cases of viral gastroenteritis.[5,6,7,8,9] Rapid and accurate diagnosis of gastroenteritis due to adenovirus is helpful in establishing the etiology of gastroenteritis and related patient management. Other diagnostic techniques such as electron microscopy (EM) and nucleic acid hybridization are expensive and labor-intensive. With the self-limiting nature of adenovirus infection, such expensive and labor-intensive tests may not be necessary.

【PRINCIPLE】

Norovirus Rapid Test is a qualitative, lateral flow immunoassay for the detection of Norovirus in human feces specimens. The assay uses Genogroup 1 and Genogroup 2 specific monoclonal antibodies coated on the test membrane. During testing, the feces 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 zone 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 zone (C) indicating that proper volume of specimen has been added and membrane wicking has occurred.

Rotavirus and Adenovirus Combo Rapid Test is a qualitative, lateral flow immunoassay for the detection of rotavirus and adenovirus in human feces specimen. In this test, the membrane is pre-coated with anti-rotavirus antibody on the T2 test line region of the test and anti-adenovirus antibody on the T1 test line region of the test. During testing, the specimen reacts with the particle coated with anti-rotavirus antibody and anti-adenovirus antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-rotavirus antibody and anti-adenovirus antibody on the membrane and generate a colored line. The presence of these colored lines in test line region indicates a positive result, while their absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

Norovirus Rapid Test

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

Rotavirus and Adenovirus Combo Rapid Test

The test contains anti-rotavirus antibody and anti-adenovirus antibody coated particles and anti-rotavirus antibody anti-adenovirus antibody coated on the membrane.

【PRECAUTIONS】

- For professional *in vitro* diagnostic use only. Do not use after the 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 pouches 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】

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, rotavirus and adenovirus in the feces of patients with gastroenteritis occurs 3-5 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 feces 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.

【MATERIALS】

Materials Provided

- Test Cassettes
- Package Insert
- Droppers
- Specimen collection tubes with extraction buffer

Materials Required But Not Provided

- Specimen collection containers
- Timer
- Centrifuge and pipette to dispense 80 µL if required

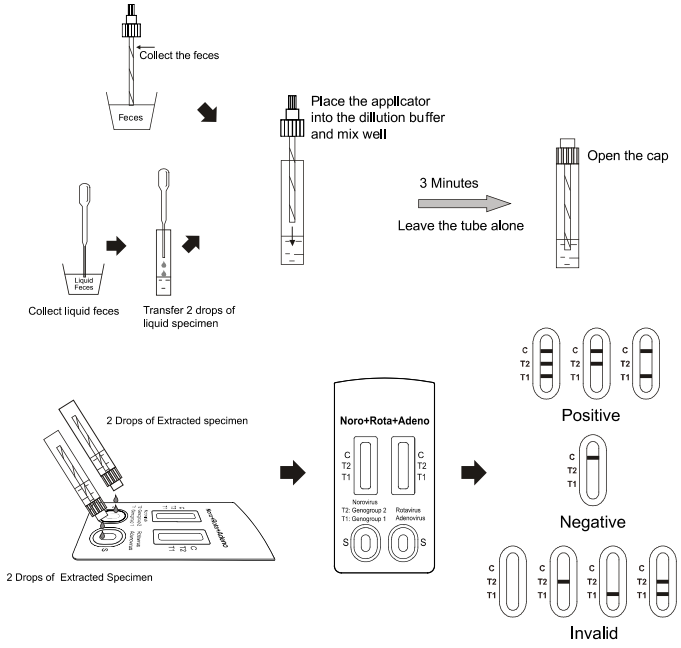
【DIRECTIONS FOR USE】

Allow the test cassette, 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 **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. Leave the collection tube for reaction for 3 minutes.
3. 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 **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 each specimen well of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen wells. 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】

(Please refer to the illustration above)

Norovirus Window:

Genogroup 1&2 POSITIVE:* Three colored lines appear. One colored line should be in the control line region (C) and colored lines should be in the Genogroup 1 region (T1) and Genogroup 2 region (T2).

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

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

***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.

Rota/Adeno Window:

Rotavirus Positive: * A colored line appears in the control line region (C) and another colored line appears in the T2 line region.

Adenovirus Positive: * A colored line appears in the control line region (C) and another colored line appears in the T1 line region.

Rotavirus and Adenovirus Positive: * A colored line appears in the control line region (C) and two other colored lines appear in T1 line region and T2 line region respectively.

***NOTE:** The intensity of the color in the test line region (T1/T2) will vary depending on the concentration of rotavirus or adenovirus antigens present in the specimen. Therefore, any shade of color in the test line region (T1/T2) 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 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. The Norovirus/Rotavirus/Adenovirus Combo Rapid Test Cassette (Feces) is for *in vitro* diagnostic use only. The test should be used for the detection of human Norovirus, Rotavirus and Adenovirus in fecal specimen only. Neither the quantitative value nor the rate of increase in human norovirus, rotavirus and adenovirus concentration can be determined by this qualitative test.
2. The Norovirus/Rotavirus/Adenovirus Combo Rapid Test Cassette (Feces) will only indicate the presence of norovirus, rotavirus and adenovirus in the specimen and should not be used as the sole criteria for the conforming norovirus, rotavirus and adenovirus to be etiological agent for diarrhea.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of norovirus, rotavirus or adenovirus infection with low concentration of virus particles.

【PERFORMANCE CHARACTERISTICS】

Clinical Sensitivity, Specificity and Accuracy

1. Norovirus

The performance of the Norovirus Rapid Test Cassette has been evaluated with 136 clinical specimens. The results show that the relative sensitivity of the Norovirus Rapid Test Cassette (Feces) is ＞99.9% and the relative specificity is 98.1%.

Norovirus Rapid Test Cassette vs. other test

Method		Other test		Total Results
Norovirus Rapid Test Cassette	Results	Positive	Negative	
	Positive	33	2	35
	Negative	0	101	101
Total Results		33	103	136

Relative Sensitivity: ＞99.9% (95%CI:*91.32%-99.92%) *Confidence Intervals
Relative Specificity: 98.1% (95%CI:*93.16%-99.76%)
Relative Accuracy: 98.5% (95%CI:*94.79%-99.82%)

2. Rotavirus

The performance of the Rotavirus Rapid Test Cassette has been evaluated with 501 clinical specimens collected from children and young adults in comparison with latex agglutination method. The results show that the relative sensitivity of the Rotavirus Rapid Test Cassette (Feces) is 97.3% and the relative specificity is 97.1%.

Method		Latex Agglutination		Total Results
Rotavirus rapid test Cassette	Results	Positive	Negative	
	Positive	251	7	258
	Negative	7	236	243
Total Results		258	243	501

Relative Sensitivity: 97.3% (95%CI:*94.5%-98.9%) *Confidence Intervals
Relative Specificity: 97.1% (95%CI:*94.2%-98.8%)
Relative Accuracy: 97.2% (95%CI:*95.4%-98.5%)

3. Adenovirus

The performance of the Adenovirus Rapid Test Cassette has been evaluated with 381 clinical specimens collected from children and young adults in comparison with latex agglutination method. The results show that the relative sensitivity of the Adenovirus Rapid Test Cassette (Feces) is 95.2% and the relative specificity is 97.7%.

Method		Latex Agglutination		Total Results
Adenovirus rapid test	Results	Positive	Negative	
	Positive	118	6	124
	Negative	6	251	257
Total Results		124	257	381

Relative Sensitivity: 95.2% (95%CI:*89.8%-98.2%) *Confidence Intervals
Relative Specificity: 97.7% (95%CI:*95.0%-99.1%)
Relative Accuracy: 96.8% (95%CI:*94.6%-98.4%)

Precision

Intra-Assay

Within-run precision has been determined by using 3 replicates of three different specimens containing different concentrations of norovirus, rotavirus and adenovirus antigen. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 3 independent assays on the same different specimens containing different concentrations of norovirus, rotavirus and adenovirus antigen. Three different lots of the Norovirus/Rotavirus/Adenovirus Combo Rapid test have been tested over a 3-days period using above negative and positive specimens. 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	*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*

Cross reactivity with following organisms has been studied at 1.0×10^9 organisms/mL. The following organisms were found negative when tested with the Rotavirus/Adenovirus Combo Rapid test Cassette (Feces).

Staphylococcus aureus	*Proteus mirabilis*	*Neisseria gonorrhea*
Pseudomonas aeruginosa	*Acinetobacter spp*	*Group B Streptococcus*
Enterococcus faecalis	*Salmonella choleraesius*	*Proteus vulgaris*
Group C Streptococcus	*Gardnerella vaginalis*	*Enterococcus faecium*
Klebsiella pneumoniae	*Acinetobacter calcoaceticus*	*Hemophilus influenzae*
Branhamella catarrhalis	*E.coli*	*Neisseria meningitidis*
Candida albicans	*Chlamydia trachomatis*	

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Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged		Manufacturer		Consult Instructions For Use

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