

Rotavirus Rapid Test Cassette
(Feces)
Package Insert

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

【INTENDED USE】
TheRotavirus Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of rotavirus in human feces specimens to aid in the diagnosis of rotavirus infection.

【SUMMARY】
Rotavirus is the most common agent responsible for acute gastroenteritis, mainly in young children. Its discovery in 1973 and its association with infantile gastro-enteritis represented a very important advancement in the study of gastro-enteritis not caused by acute bacterial infection. Rotavirus is transmitted by oro-faecal 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 diarrhoea continues. Rotaviral gastroenteritis may result in mortality for populations at risk such as infants, the elderly, and immunocompromised patients.² In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported.³ With hospitalised children suffering from acute enteric disease up to 50% of the analysed specimen were positive for rotavirus.⁴ 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.

TheRotavirus Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of rotavirus in human feces specimen, providing results in 10 minutes. The test utilizes antibody specific for rotavirus to selectively detect rotavirus from human feces specimens.

【PRINCIPLE】
TheRotavirus Rapid Test Cassette (Feces) is a qualitative, lateral flow immunoassay for the detection of rotavirus in human feces specimens. In this test, the membrane is pre-coated with anti-rotavirus antibody on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-rotavirus antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-rotavirus antibody on the membrane and generate a colored line in the test line region. The presence of this colored line in the test region indicates a positive result, while its 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】
The test contains anti-rotavirus antibody coated particles and anti-rotavirus antibody 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 rotavirus 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.
- The feces specimen must be collected in a 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

Specimen collection tubes with extraction buffer

Package insert

Droppers

Materials Required But Not Provided

Specimen collection containers

Centrifuge and pipette to dispense 80 µL if required

Timer

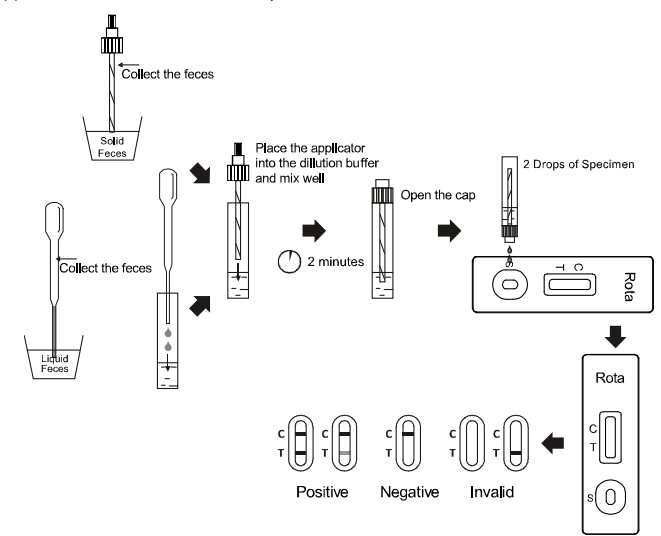
【DIRECTIONS FOR USE】
Allow the test, specimen, buffer, and/or controls to reach room temperature (15-30°C) prior to testing.

- 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.
- To process fecal specimens:
 - ForSolid 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.

- ForLiquid 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 2 minutes.
 - 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.
 - Hold the specimen collection tube upright and **unscrew the tip** 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.
 - Read the results at **10 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)

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 test line region (T).

***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of rotavirus 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】**
- TheRotavirus Rapid Test Cassette (Feces) is for *in vitro* diagnostic use only. The test should be used for the detection of human rotavirus in feces specimens only. Neither the quantitative value nor the rate of increase in human rotavirus concentration can be determined by this qualitative test.
 - TheRotavirus Rapid Test Cassette (Feces) will only indicate the presence of rotavirus in the specimen and should not be used as the sole criteria for the conforming rotavirus to be etiologic agent for diarrhea.
 - As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
 - 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 rotavirus infection with low concentration of virus particles.

【EXPECTED VALUES】
TheRotavirus Rapid Test Cassette (Feces) has been compared with latex agglutination method, demonstrating an overall accuracy of 97.2%.

【PERFORMANCE CHARACTERISTICS】
Clinical Sensitivity, Specificity and Accuracy
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%.

Rotavirus Rapid Test Cassette vs. Latex Agglutination				
Rotavirus Rapid Test Cassette	Method	Latex Agglutination		Total Results
	Results	Positive	Negative	
		251	7	258
	Positive	251	7	258
	Negative	7	236	243
	Total Results	258	243	501

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

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 using 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 10⁹ organisms/mL. The following organisms were found negative when tested with the Rotavirus Rapid Test Cassette (Feces).
Staphylococcus aureus
Pseudomonas aeruginosa
Enterococcus faecalis
Group C Streptococcus
Klebsiella pneumoniae
Branhamella catarrhalis
Candida albicans
Proteus mirabilis
Acinetobacter spp
Salmonella choleraesuis
Gardnerella vaginalis
Acinetobacter calcoaceticus
E. coli
Chlamydia trachomatis
Neisseria gonorrhea
Group B Streptococcus
Proteus vulgaris
Enterococcus faecium
Hemophilus influenzae
Neisseria meningitidis

Interfering Substances
The following potentially interfering substances were added to Rotavirus negative and positive specimens.
Ascorbic acid: 20mg/dl
Uric acid: 60mg/dl
Glucose: 2000mg/dl
Oxalic acid: 60mg/dl
Aspirin: 20mg/dl
Caffeine: 40mg/dl
Bilirubin: 100mg/dl
Urea: 2000mg/dl
Albumin: 2000mg/dl

【BIBLIOGRAPHY】

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Number:
Effective date: