ROTADENO COMBO DEVICE (2-30°C)

CATALOGUE NUMBER	KIT SIZE (TESTS)
RADRDN1	20 Tests

Intended Use:
The Rotadeno Combo Rapid Test Device (Faeces) is a rapid chromatographic immunoassay for the qualitative presumptive detection of rotavirus and adenovirus antigens in human faecal samples. This kit is intended to be used as an aid in the diagnosis of rotavirus and adenovirus infection.

Summary:
Acute diarrhoea disease in young children is a major cause of morbidity worldwide and is a leading cause of mortality in developing countries. 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. In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported. With hospitalised children suffering from acute enteric disease up to 50% of the analysed specimen were positive for rotavirus. 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 faeces.
Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhoea in many of these children, second only to the rotaviruses. These viral pathogens have been isolated throughout the world and can cause diarrhoea 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.
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 labour-intensive. With the self-limiting nature of adenovirus infection, such expensive and labour-intensive tests may not be necessary.

Test Principle:
The Rotadeno Combo Rapid Test Device (Faeces) is a qualitative, lateral flow immunoassay for the detection of rotavirus and adenovirus antigens in human faecal samples.
In this test, the membrane is pre-coated with anti-rotavirus antibody on the T1 test line region of the test and anti-adenovirus antibody on the T2 test line region. During testing the sample, if it contains antigens, reacts with particles coated with anti-rotavirus antibody and anti-adenovirus antibody. The mixture migrates upward on the membrane chromatographically by capillary action where the antigens also react with and are captured by anti-rotavirus antibody and anti-adenovirus antibody coated on the membrane to generate a coloured line. The presence of these coloured lines in test line region indicates a positive result, while their absence indicates a negative result. To serve as a procedural control, a coloured line should 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 and anti-adenovirus antibody coated particles and anti-rotavirus antibody and anti-adenovirus antibody coated on the membrane.

Materials Provided
Individually pouched test devices
Specimen collection tubes with extraction buffer
Droppers
Instructions for Use sheet

Materials not provided: Timer, specimen collection container, centrifuge and pipette to dispense 80µl if required

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

Storage and Stability:
Store as packaged in the sealed pouches either at room temperature or refrigerated (2-30°). The test is stable until the expiry 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 expiry date.

Specimen Collection and Preparation:
- Viral detection is improved by collecting the samples at the onset of the symptoms. It has been reported that the maximum excretion of rotavirus and adenovirus in the faeces of patients with gastroenteritis occurs 3-5 days after onset of symptoms. If the specimens are collected long after the onset of diarrhoeic symptoms, the quantity of antigen may not be sufficient to obtain a positive reaction or the antigens detected may not be linked to the diarrhoeic episode.
- The faeces sample must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
- Bring the necessary reagents to room temperature before use.

Assay Procedure:
Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

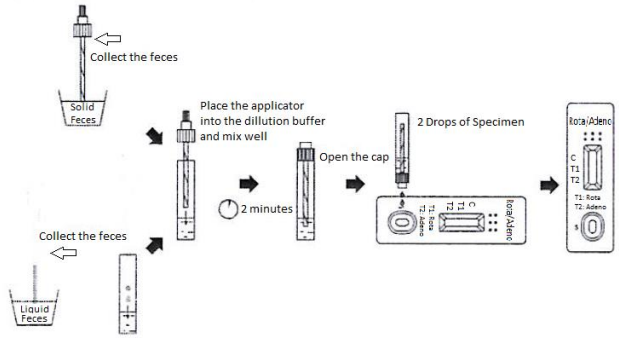
1. Sample collection and pre-treatment:

1) Collect sufficient quantity of faeces (1-2ml or 1-2g) 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. Samples collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long-term storage, samples should be kept below -20°C.

2. To process faecal samples:

2) **For solid specimens:** Unscrew the cap of the specimen collection tube, then randomly stab the sample collection applicator into the faecal sample in at least 3 different sites to collect approximately 50mg of faeces (equivalent to ¼ of a pea). Do not scoop the faecal sample.
3) **For liquid specimens:** Hold the dropper vertically, aspirate faecal samples, and then transfer 2 drops of the liquid sample (approximately 50µl) into the specimen collection tube containing the extraction buffer.
Cap the specimen collection tube, then shake the tube vigorously to mix the sample and the extraction buffer. Leave the collection tube to stand for 2 minutes.
4) Bring the pouch to room temperature before opening it. Remove the test device 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.
5) Hold the specimen collection tube upright and open the cap to reveal the dropper system. Invert the specimen collection tube and transfer 2 full drops of the extracted sample (approximately 80µl) to the sample well (S) of the test device, then start the timer. Avoid trapping air bubbles in the sample well (S).
6) Read the results at 10 minutes after dispensing the sample. Do not read results after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the extracted specimens contained in the extraction buffer vial. Collect 80 µL of supernatant, dispense into the specimen well (S). Start the timer and continue from the step 6 onwards.



Interpretation of Results:

POSITIVE RESULT:

Rotavirus Positive: * A coloured band appears at the control line (C) and another coloured band appears in the (T1) line region.

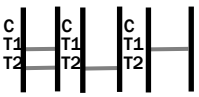
Adenovirus Positive: * A coloured band appears at the control line (C) and another coloured band appears in the (T2) line region.

Rotavirus and Adenovirus Positive: * A coloured band appears at the control line (C) and two other coloured bands appear in (T1) line region and (T2) line region respectively.

NEGATIVE RESULT:

Only one coloured band appears, in the control line (C). No coloured band appears in (T1) line region or (T2) line region.

INVALID RESULT:



Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:
1. The intensity of colour in (T2) line region and (T1) line region may vary depending on the concentration of analytes present in the sample. Therefore, any shade of

colour in the test region should be considered positive. Note that this is a qualitative test only and cannot determine the concentration of analytes in the sample.

Quality Controls:

- A procedural control is included in the test. A line appearing in the control line region (C) is the internal procedural control. It confirms sufficient sample volume was added and correct procedural technique.
- Quality Controls are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations of the Test:

1. The Rotadeno Combo Rapid Test Device (Faeces) is for professional *in vitro* diagnostic use and should be used for the qualitative detection of rotavirus and adenovirus only. Neither the quantitative value nor the rate of increase of rotavirus and adenovirus concentration can be determined by this test.
2. The Rotadeno Combo Rapid Test Device (Faeces) will only indicate the presence of rotavirus and adenovirus in the specimen and should not be used as the sole criteria for the conforming rotavirus and adenovirus to be etiological agent for diarrhoea.
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 rotavirus infection or adenovirus infection with low concentration of virus particles.

Expected Values:

The Rotadeno Combo Test Device (Faeces) has been compared with latex agglutination methods, demonstrating an overall accuracy of ≥ 97.0%.

Performance Characteristics:

Clinical Sensitivity, Specificity and Accuracy

The performance of the Rotadeno Combo Rapid Test Device (Faeces) has been evaluated the clinical specimens collected from children and young adults in comparison with latex agglutination methods. The results show that the Rotadeno Combo Rapid Test Device (Faeces) has high sensitivity and specificity for rotavirus and adenovirus.

Rotavirus results:

Method		Latex Agglutination		Total Results
Rotadeno Combo Rapid Test Device	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%)
Relative Specificity: 97.1% (95%CI*: 94.2% - 98.8%)
Relative Accuracy: 97.2% (95%CI*: 95.4% - 98.5%) *Confidence Intervals

Adenovirus results:

Method		Latex Agglutination		Total Results
Rotadeno Combo Rapid Test Device	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%)
Relative Specificity: 97.7% (95%CI*: *95.0% - 99.1%)
Relative Accuracy: 96.8% (95%CI*: 94.6 – 98.4%) *Confidence Intervals

Precision

Intra-Assay

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

Inter-Assay

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

Cross-Reactivity

Cross reactivity with following organisms has been determined at a level of 1.0 x 10^9 organisms/ml. The following organisms were found negative when tested with the Rotadeno Combo Rapid Test Device (Faeces).

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

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GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		Do not reuse

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