INSTRUCTIONS FOR USE

ONE STEP TEST

Rotavirus and Adenovirus Detection in Feces

Only for professional *in vitro* diagnostic use.

Product Code : TRA01

Rotavirus Adenovirus Combo Test Device qualitatively detects Rotavirus and Adenovirus antigens in human feces.

INTENDED USE

Rotavirus Adenovirus Combo Test Device is a rapid immunochromatographic assay for qualitative detection of Rotavirus and Adenovirus antigens in human feces samples to aid in the diagnosis of Rotavirus and Adenovirus infection.

BACKGROUND INFORMATION

Acute diarrhea 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 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. In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported. With hospitalized children suffering from acute enteric disease up to 50% of the analyzed 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 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. 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. 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 36acid hybridization are expensive and labor-intensive. With the self-limiting nature of adenovirus infection, such expensive and labor-intensive tests may not be necessary.

REAGENTS

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

METHOD

Rotavirus Adenovirus Combo Test Device is a qualitative, immunochromatographic assay for detection of Rotavirus and Adenovirus in human feces samples. "R" test area of this test is pre-coated with anti-rotavirus antibodies and "A" test area of this test is pre-coated with anti-adenovirus antibodies. While performing the test; sample dropped to the sample well reacts with the particles coated with anti-rotavirus antibodies and/or anti-adenovirus antibodies. This complex migrates to the other end of the membrane by capillary action. If there is Rotavirus in the sample, they bind to anti-rotavirus antibodies in the "R" test area and create a visible, colored signal that means the test is result is positive. If there is Adenovirus in the sample, they bind to anti-adenovirus antibodies in the "A" test area and create a visible, colored signal that means the test is result is positive. If the sample does not contain Rotavirus and/or Adenovirus, colored lines does not appear in the "R" and "A" test areas. This means the test result is negative. As a procedural control, colored line always appears in the "C" control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1.For professional and *in vitro* diagnostic use only.
2.Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3.The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4.Wear disposable gloves while performing the test.
5.Use a new dropper for each sample.
6.All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7.This test will indicate only the presence or absence of Rotavirus and Adenovirus antigens in the sample, and should not be used as the only basis for the confirming rotavirus and adenovirus to be etiological agent for diarrhea.
As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.
8.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 and Adenovirus infection.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources.
Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

Kit components : Test devices, droppers, sample collection tubes with extraction buffer and instructions for use.

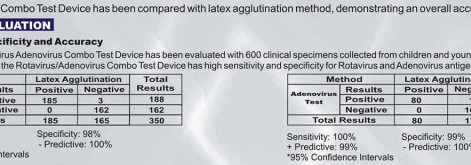
Additional materials required but not provided : Sample collection containers, centrifuge and timer.

Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

TEST PROCEDURE

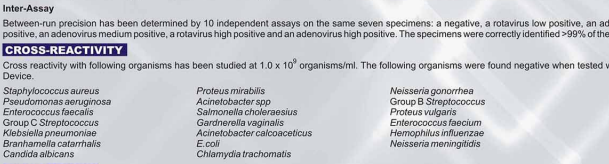
Take the test device out of its pouch. Bring the tests, dilution buffer and samples to room temperature.
1.**Feces samples :**
Feces sample must be collected in clean, dry, waterproof container containing no detergents, preservatives and transport media. Take 1 - 2 ml or 1- 2 g feces sample to the container to collect sufficient quantity of antigen (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Collected samples may be stored 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 fecal samples :**
a. For solid samples;
Unscrew the cap of the sample collection tube. Stab the sample collection applicator randomly into the fecal sample in at least 3 different sites to collect approximately 50 mg of feces. Screw the applicator to the sample collection tube with the sample on it.
b. For liquid samples;
Hold the dropper vertically and draw feces sample into the dropper. Put 2 drops (approximately 50 µl) of sample in the sample collection tube.
3.Screw the cap of the sample collection tube and shake well to mix the sample and the dilution buffer. Wait for two minutes.
4.Hold the sample collection tube upright and break off the tip. Transfer 2 drops of extracted sample (approximately 80 µl) to the sample well of the cassette. Avoid the formation of any air bubbles.
5.Depending on the Rotavirus antigen and Adenovirus antigen concentration in the sample, the test can react even in 5 minutes. Results should be read at 10 minutes as shown below. Results forming after 20 minutes should be regarded as invalid.

NOTE : If the extracted sample does not migrate in the test because of the particles, centrifuge the extracted sample in the sample collection tube. Then collect 80 µl supernatant and dispense it to the sample well of a new test device and follow the instruction from step 5.



INTERPRETATION OF RESULTS

Negative : Only one colored line is visible in "C" area, indicating that Rotavirus and Adenovirus antigens do not exist.
Positive :
Rotavirus Positive : Two colored lines are visible in "C" and "R" areas, indicating that rotavirus antigen exists.
Adenovirus Positive : Two colored lines are visible in "C" and "A" areas, indicating that adenovirus antigen exists.
Rotavirus and Adenovirus Positive : Colored lines are visible in "C", "R" and "A" areas, indicating that rotavirus and/or adenovirus antiges exist.
Low concentration of rotavirus antigen and/or adenovirus antigen may cause a faint line in "R" and/or "A" areas. Even such a faint line in "R" and/or "A" area should be regarded as "positive".
Invalid : No colored line is visible in "C" area; test should be repeated using a new test device.



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "R" and/or "A" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

EXPECTED VALUES

The Rotavirus Adenovirus Combo Test Device has been compared with latex agglutination method, demonstrating an overall accuracy of >99.0%.

PERFORMANCE EVALUATION

Clinical Sensitivity, Specificity and Accuracy

The performance of the Rotavirus Adenovirus Combo Test Device has been evaluated with 600 clinical specimens collected from children and young adults in comparison with latex agglutination method. The results show that the Rotavirus/Adenovirus Combo Test Device has high sensitivity and specificity for Rotavirus and Adenovirus antigens.

Method		Latex Agglutination		Total Results
Rotavirus Test	Results	Positive	Negative	
	Positive	185	3	188
	Negative	0	162	162
Total Results		185	165	350

Sensitivity: 100%
+ Predictive: 98%
*95% Confidence Intervals
Specificity: 98%
- Predictive: 100%

Method		Latex Agglutination		Total Results
Adenovirus Test	Results	Positive	Negative	
	Positive	80	1	81
	Negative	0	169	169
Total Results		80	170	250

Sensitivity: 100%
+ Predictive: 99%
*95% Confidence Intervals
Specificity: 99%
- Predictive: 100%

Intra-Assay

Within-run precision has been determined by using 10 replicates of seven specimens: 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 specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same seven specimens: 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 specimens were correctly identified >99% of the time.

CROSS-REACTIVITY

Cross reactivity with following organisms has been studied at 1.0 x 10^9 organisms/ml. The following organisms were found negative when tested with the Rotavirus Adenovirus Combo Test Device.

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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SYMBOLS USED

CE
EC REP Manufacturer
Authorized European Representative
Attention, see instruction for use
Consult instruction for use
IVD *In vitro* diagnostic medical device
Number of test
For single use only
Storage temperature
LOT Lot number
REF Catalog number
Expiry date