INSTRUCTIONS FOR USE

ONE STEP TEST
Rotavirus Antigen
Detection in Feces

TOYO DIAGNOSTICS

Only for professional *in vitro* diagnostic use

Product Code: TROV01
Rotavirus Test Device detects Rotavirus antigens in human feces.

INTENDED USE

Rotavirus Test Device is a rapid immunochromatographic assay for the qualitative detection of rotavirus antigens in human feces samples.

BACKGROUND INFORMATION

Rotavirus has been recognized for 30 years as the most common cause of infectious gastroenteritis in infants and young children. By contrast, the role of rotavirus as a pathogen in adults has long been underappreciated. Spread by fecal-oral transmission, rotavirus infection in adults typically manifests with nausea, malaise, headache, abdominal cramping, diarrhea, and fever. Infection can also be symptomless.
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. Specimen collections taken within the second and fifth days of the illness are ideal for antigen detection, 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 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 human feces.

REAGENTS

The test contains coated particles with anti-rotavirus antibodies and anti-rotavirus antibodies immobilized on the membrane.

METHOD

Rotavirus Test Device is a rapid, qualitative, immunochromatographic assay for detection of rotavirus in human feces samples.
There are anti-rotavirus antibodies immobilized on the "T" test area of this test. While performing the test; sample dropped to the sample well reacts with the particles coated with anti-rotavirus 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 "T" test area and create a visible, colored signal that means the test result is positive. If the sample does not contain rotavirus, colored line does not appear in the "T" test area. 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 antigen in the sample, and should not be used as the only basis for the diagnosis of Rotavirus infection.
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 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 dilution buffer and instructions for use.

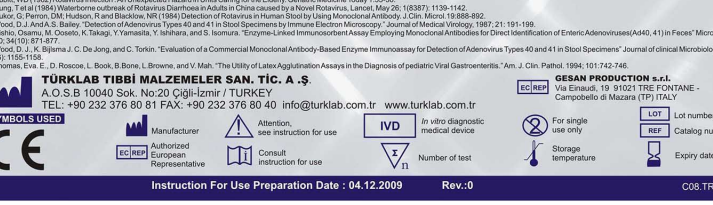
Additional materials required but not provided : Sample collection containers, centrifuge and timer.

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

TEST PROCEDURE

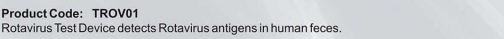
Take the test device out of its pouch. Bring the tests, dilution buffer and samples to room temperature.
1. Feces sample :
Feces sample must be collected in clean, dry, waterproof container containing no detergents, preservatives and transport media. Take 1 - 2 ml or 1 - 2 g feces sample to the container to collect sufficient quantity of rotavirus 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 :
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.
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 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 antigen does not exist.
Positive : Two colored lines are visible in "C" and "T" areas, indicating that Rotavirus antigen exists.
Low concentration of Rotavirus antigen may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid : No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.



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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 "T" 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.

PERFORMANCE EVALUATION

Rotavirus Test Device has been evaluated with clinical samples from children and young adults. Latex agglutination method is used as a reference and following results obtained.

Sensitivity : 99,99 % **Specificity : 99 %** **+ Predictive V : 99 %** **- Predictive V : 99,99 %**
Confidence interval : 95 %

		Reference	
		+ Result	- Result
Test	+ Result	158	2
	- Result	0	140

Intra Assay
Within-run precision of the same test has been confirmed with 100 replicates of negative, Rotavirus low positive, Rotavirus medium positive and Rotavirus high positive samples. Negative, Rotavirus low positive, Rotavirus medium positive and Rotavirus high positive values were correctly determined for each trial.

Inter Assay
Between-run presicion of the same test has been confirmed with 10 independent assays with the same negative, Rotavirus low positive, Rotavirus medium positive and Rotavirus high positive samples. Negative, Rotavirus low positive, Rotavirus medium positive and Rotavirus high positive values were correctly determined for each trial.

CROSS REACTIVITY

Cross reactivity has been tested with below samples (1,0 X 10^9 microorganism/ml), no cross reactivity was found with the Rotavirus 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 calcoacetius
E.coli
Chlamydia trachomatis
Neisseria gonorrhea
Group B Streptococcus
Proteus vulgaris
Enterococcus faecium
Hemophilus influenzae
Neisseria meningitidis

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SYMBOLS USED

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