SAS™
Rota Test

A RAPID TEST FOR THE QUALITATIVE DETECTION OF
ROTAVIRUS ANTIGENS
IN HUMAN FECAL SPECIMENS

For *In-Vitro* Diagnostic Use

Store at 15°C to 30°C

For Technical Assistance Call 800-272-2710
Outside the USA Call 210-699-8800

SA Scientific™

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INTENDED USE

SAS™ Rota Test is a rapid, membrane-based immunogold assay for the qualitative detection of Rotavirus antigens in feces as an aid in the diagnosis of acute gastroenteritis caused by Rotavirus infection. This test is for professional use only.

SUMMARY AND EXPLANATION

Rotavirus is the major cause of life-threatening diarrhea (gastroenteritis) in children younger than 2 years[1,2]. Rotavirus gastroenteritis is ubiquitous, it occurs in all parts of the world[1,2]. These double stranded RNA viruses cause epidemic and endemic gastroenteritis in pediatric as well as geriatric patients[3]. It afflicts more than 130 million infants and children annually, causing 1 million deaths[2].

The virus infects the epithelial cells lining the small intestine[1]. After an incubation period of 1-2 days, the onset of acute gastroenteritis is sudden, with vomiting and diarrhea, fever, occasional abdominal pain, and even respiratory symptoms[4]. The duration of the disease and the symptoms may last between 5-8 days.

The gold standard for detecting Human Rotavirus depends on electron microscopy[5] since existing tissue culture methods are unreliable. The SAS™ Rota Test offers a fast, simple method for the detection of Rotavirus and Rotavirus antigens.

PRINCIPLE OF THE TEST

The SAS™ Rota Test utilizes a pair of Rotavirus-specific antibodies in an immunochromatographic sandwich assay. An extract is first prepared by suspension of the specimen in the provided extraction buffer solution. The buffer containing the extracted specimen is then added to the device's sample well. The reaction between a positive sample and the colored particle-conjugated antibody will form a complex that migrates along the membrane. An immobilized capture antibody will form a colored line at the S (specimen) area upon reacting with the colored complex. An internal control line C (control) area is built-in to assure that the test has been carried out correctly.

MATERIALS AND REAGENTS PROVIDED

1. Test devices contain a test strip with a monoclonal anti-Rotavirus antibody, colored conjugate, and polyclonal antibody coated on a membrane. The monoclonal antibody is affinity purified and is specific to the capsid protein of Rotavirus.
2. Tubes containing extraction buffer – Buffer contains 0.1% sodium azide.
3. Disposable sample transfer pipettes.
4. Package Insert.

MATERIALS NOT PROVIDED

1. Sterile specimen containers.
2. Vortex or centrifuge.
3. Timer.
4. Rotavirus positive control.
5. Rotavirus negative control.

PRECAUTIONS

1. For *in-vitro* diagnostic use only.
2. The test device should remain in the sealed pouch until ready for use.
3. Do not mouth pipette samples.
4. Do not smoke, eat or drink in areas where specimens or kit components are handled.
5. All specimens, reagents, and controls should be considered potentially hazardous and handled in the same manner as an infectious agent.
6. Wear disposable gloves while handling samples and wash hands after the assay is complete. Warning: The user should refer to the relevant section of the CDC-NIH manual "Biosafety in Microbiology and Biomedical Laboratories," 3rd Edition, 1984.
7. Avoid any contact with the eyes, broken skin, or mucous membranes.
8. Avoid splashing or the generation of aerosols.
9. The test device and all materials should be discarded in a proper biohazard container after testing.
10. Do not use kit or materials beyond expiration date.
11. Do not mix or interchange lots of SAS™ Rota Test devices or reagents.
12. Extraction buffer contains sodium azide, which may react with lead and copper plumbing to form explosive metal azides. Azide build-up may be avoided by flushing drains with large volumes of water after disposal.
13. Avoid microbial contamination of reagents or incorrect results may occur. Contamination of samples could cause false results.
14. Use separate pipettes or pipette tips for each sample, control and reagent.
15. Do not reuse test devices or kit materials.

STORAGE INSTRUCTIONS

The test kit is to be stored at room temperature (15° - 30°C) for the duration of the shelf life. The test device must remain in the sealed pouch until ready for use.

SPECIMEN COLLECTION & PREPARATION

Specimens should be collected as soon as possible after the onset of symptoms. It is recommended that the specimen be collected during the acute phase of gastroenteritis, because a large number of viral particles and viral antigens are excreted during this period.

Peak viral counts usually occur 3-5 days after the onset of symptoms. Samples collected more than 7 days after the onset of symptoms may not contain enough Rotavirus antigen to produce a positive reaction.

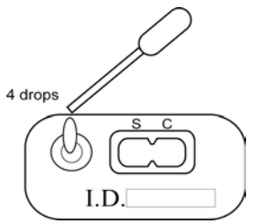
The specimen should be collected in a clean, dry container, either plastic or glass. Do not collect specimens in containers having media, animal serum, detergents or preservatives, as these may interfere with the assay. If necessary, a sample can be collected from a soiled diaper of young children and neonates. Do not freeze samples unless a delay in testing is expected. In this case, quickly freeze samples using dry ice, and keep sample frozen at -20°C or colder until ready for testing.

TEST PROCEDURE

Allow the pouch (test device), specimen and/or controls to reach room temperature (15° - 30°C) prior to testing. Swabs or samples must be extracted using the provided SAS™ extraction buffer. Rub the swab carefully against the tube containing extraction buffer.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with patient or control identifications.
2. Using a swab, add a sufficient stool sample (30-50mg) to the tube containing the SAS™ Extraction Buffer.
3. Rub the swab meticulously against the inner wall of the tube in order to release the fecal material.
4. For best results, centrifuge the tube containing the sample for 1-2 minutes.
5. Using the sample transfer pipettes provided, dispense 4 drops (approximately 150µl) of the supernatant into the round sample well of the device (see illustration below). Wait for colored lines to appear.

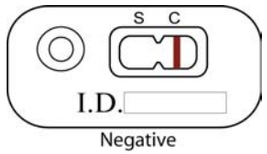
6. Read results at 15 minutes. Some positive results may be observed in as short as 30 seconds depending on the concentration of antigen. Do not interpret results after 15 minutes.



INTERPRETATION OF RESULTS

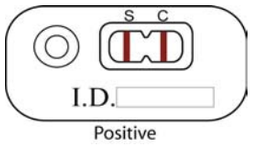
Negative Results

The test is negative if only one colored line appears in the C (control) area.



Positive Results

The test is positive if two colored lines appear. One colored line will appear in the S (specimen) area and one in the C (control) area. Any colored line in the S area should be considered positive. Colored lines may be lighter or darker than each other.



Invalid Results

The test is invalid if no colored line appears in the C (control) area, even if a colored line appears in the S (specimen) area. If this occurs, add 1 to 2 additional drops of sample and wait for 5 minutes. If a colored line does not appear in the C area, the test is invalid and should be repeated. Colored lines that appear after 15 minutes are not diagnostic and should be ignored.

QUALITY CONTROL

Internal Controls

Each test device includes internal procedural controls. The appearance of a Control Line in the C region of the test device is a positive procedural control. Correct procedural technique, specimen flow and test device performance is confirmed when a colored line appears in the C (control) area of the membrane. If the colored line fails to appear in the C (control) area, the test result is invalid.

A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. If a more intensely red background color appears, it may interfere with the ability to read the test result, therefore the test should be repeated.

The procedural controls do not test for the presence or absence of Rotavirus. A positive control containing inactivated Rotavirus can be used as a control.

External Controls

It is recommended that when a new shipment of product is received, negative and positive controls for Rotavirus should be tested and the appropriate results obtained (See NCCLS C24-A for guidance on appropriate quality control practices).

LIMITATIONS OF THE PROCEDURE

1. SAS™ Rota Test is highly sensitive and specific for Rotavirus antigen. The monoclonal antibody in this test will detect human Rotaviruses, but cannot be used to differentiate types.
2. The test is highly dependent on the collection and preparation of the clinical specimen. Care should be taken to adhere to proper procedures.
3. A negative result does not exclude the possibility of Rotavirus infection in the patient. False negative results may occur due to low concentration levels of the Rotavirus antigen below the sensitivity level of the test, improper or inadequate sampling, or improper handling of the specimen.
4. Co-infection with bacterial pathogens is possible. Therefore, bacteriological tests should be performed in parallel with this test to rule out bacteriological etiology.

EXPECTED VALUES

Rotavirus infection is seasonal and is the most frequent cause of gastroenteritis in children between 6 months and 3 years of age. Among young children hospitalized for gastroenteritis, it is expected that up to 50% of patient specimens will test positive for Rotavirus[6]. The prevalence of Rotavirus infection will vary based on many factors such as age, geographic location, method of sample collection, sample handling and transportation, and the general health environment of the patient population under study. Normal healthy individuals tested should be negative for Rotavirus. Some infected individuals may show symptoms or only minor symptoms, and these patients may test negative.

PERFORMANCE CHARACTERISTICS

The SAS™ Rota Test was tested in laboratories and hospitals in the United States and Japan for the direct testing of patient stool samples. A total of 185 samples were tested, and the results compared to the Meridian Premier Rotaclone® EIA test, and to electron microscopy (EM).

	Sites I & II U.S.			Site III Japan	
Comparison	EM			Meridian Rotaclone®	
	+	–		+	–
+	50	0		13	2
SAS™ Rota Test					
–	0	50		0	70

Performance Statistic	Value	95% C.I.		Value	95% C.I.
Sensitivity	100%	92.9%-100%	Relative Sensitivity	100%	75.3%-100%
Specificity	100%	92.9%-100%	Relative Specificity	97.2%	90.3%-99.7%
Agreement	100%	96.4%-100%	Relative Agreement	97.7%	91.8%-99.7%

Note: Please be advised that "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

REPRODUCIBILITY

Three different lots of the SAS™ Rota Test were tested in multiple replicates using negative, low positive, and high positive samples. Reproducibility was tested within each lot, between lots, between different test sites, and on different days. In each case, all tests yielded 100% reproducibility.

LIMITS OF DETECTION

A study was performed in Tokyo, Japan to determine the limits of detection of the SAS™ Rota Test. The study involved using a human Rotavirus antigen (HRV) which was taken from culture and purified by ultra-centrifugation with 30% sucrose. The initial concentration of HRV was measured through spectrophotonomy, and serial dilutions were prepared and tested using the SAS™ Rota Test. Through this process, it was determined that the lower limit of detection of the test is a concentration of 24.3 ng/ml (ng of virus particles).

CROSS REACTIVITY

Common intestinal pathogens and other organisms occasionally present in feces were tested with the SAS™ Rota Test in order to verify that they did not interfere with the test and that no cross-reactivity would occur. The following organisms were tested at a concentration of 1.0 x 10^{10} organisms/ml and showed no cross-reactivity:

Microorganisms

Pseudomonas aeruginosa
Salmonella typhi
Salmonella para typhi
Salmonella enteriditis
Salmonella typhimurium

Klebsiella pneumoniae
Vibrio cholerae
Brucella abortus
Shigella dysenterae
Escherichia coli
Enterococcus faecalis
Proteus vulgaris
Citobacter freundii

Viruses

Enteric adenovirus
Calicivirus
Astrovirus

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2. Cimons, M. (1998). Rotavirus vaccine seems headed for approval, wide use. ASM News, 64:6-7.
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