OnSite® Rotavirus Ag Rapid Test

REF R0194C CE

Instructions for Use

INTENDED USE

The *OnSite* Rotavirus Ag Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of rotavirus antigen in fecal specimens. This device is intended to be used by professionals to aid in the diagnosis of infection with rotavirus.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

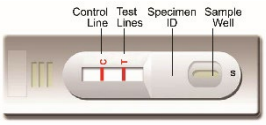
Diarrhea is one of the principle causes of childhood morbidity and mortality worldwide, resulting in 2.5 million deaths annually[1,2]. Rotavirus infection is the leading cause of severe diarrhea in infants and children under the age of five[3,4], accounting for 40%-60% of acute gastroenteritis and causing an estimated 500,000 childhood deaths each year[5]. By the age of five, nearly every child in the world has been infected with rotavirus at least once[6,7]. With subsequent infections, a broad, heterotypic antibody response is elicited; therefore, adults are rarely affected[8].

To date seven groups of rotaviruses (groups A-G) have been isolated and characterized[9]. Group A rotavirus, the most common rotavirus, causes more than 90% of all rotavirus infections in humans[10]. Rotavirus is transmitted primarily by the fecal-oral route, directly from person to person[11]. Virus titers in stool reach a maximum shortly after the onset of illness, then decline[12]. The incubation period of a rotavirus infection is usually one to three days and it is followed by gastroenteritis with an average duration of three to seven days. Symptoms of the disease range from mild, watery diarrhea to severe diarrhea with fever and vomiting.

Diagnosis of an infection with rotavirus can be made following diagnosis of gastroenteritis as the cause of severe diarrhea in children. Recently, specific diagnosis of an infection with rotavirus has become available through the detection of virus antigen in stool by immunoassay methods such as latex agglutination assay, EIA, and lateral flow chromatographic immunoassay[13,14].

The *OnSite* Rotavirus Ag Rapid Test utilizes a pair of specific antibodies to qualitatively detect the rotavirus antigen in fecal specimen. The test can be performed within 15 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Rotavirus Ag Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a colored conjugate pad containing monoclonal anti-rotavirus antibody conjugated with colloidal gold (anti-rotavirus conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with another monoclonal anti-rotavirus antibody, and the C line is pre-coated with a control line antibody.

When an adequate volume of extracted specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Rotavirus Ag, if present in the specimen, will bind to the anti-rotavirus conjugates. The immunocomplex is then captured on the membrane by the pre-coated rotavirus antibody forming a colored T line, indicating a rotavirus positive test result.

Absence of the T line suggests that the concentration of rotavirus Ag in the specimen is below the detectable level, indicating a rotavirus negative result. The test contains an internal control (C line), which should exhibit a colored line of the immunocomplex of the control antibodies, regardless of color development on the T line. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Stool collection devices, each containing 2 mL sample extraction buffer (REF SB-R0194)
3. Plastic droppers for transferring watery stool
4. Patient ID stickers
5. Instructions for Use

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container for holding test specimen

WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch until ready to conduct the assay.
3. Do not use any kit components beyond their stated expiration date.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
8. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
9. Users of this test should follow the US CDC Universal Precautions for bio-safety.
10. **Do not scoop stool sample as this may lead to excess fecal specimen that tends to clot the sample pad and interfere with sample migration.**
11. The testing results should be read 15-20 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
12. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unopened test devices at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperature over 30°C.

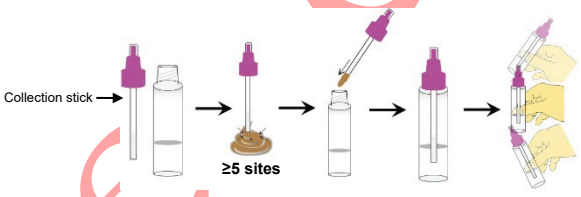
SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

To prepare specimens using solid stool samples follow Procedure A below. To prepare specimens using watery stool samples follow Procedure B below.

Procedure A: Solid stool samples

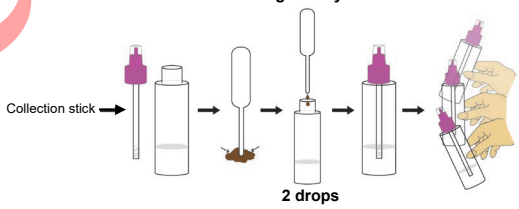
Step 1: Collect a random stool sample in a clean, dry receptacle.
Step 2: Label the stool collection device with the specimen's ID number (patient ID sticker). Open the stool collection device by unscrewing the top and use the collection stick to randomly pierce the stool sample in at least five different sites. ***Do not scoop stool sample as this may lead to an invalid test result.***
Step 3: Ensure stool sample is only in the grooves of the collection stick. ***Excess stool sample may lead to an invalid test result.***
Step 4: Replace the collection stick and tighten securely to close the stool collection device.
Step 5: **Shake the stool collection device vigorously.**



The specimen is now ready for testing, transportation or storage.

Procedure B: Watery stool samples

Step 1: Collect a random stool sample in a clean, dry receptacle.
Step 2: Label the stool collection device with the specimen's ID number (patient ID sticker). Open the stool collection device by unscrewing the top.
Step 3: Fill the plastic dropper with the sample; dispense 2 drops (70-85 µL) into the stool collection device.
Step 4: Replace the collection stick and tighten securely to close the stool collection device.
Step 5: **Shake the stool collection device vigorously.**



The specimen is now ready for testing, transportation or storage.

Note: It is recommended to test the specimen immediately after extraction. If not tested immediately, the extracted specimen may be stored at 2-8°C for up to 3 days. For longer storage, the extracted specimen may be frozen at -20°C. Avoid multiple freeze-thaw cycles.

ASSAY PROCEDURE

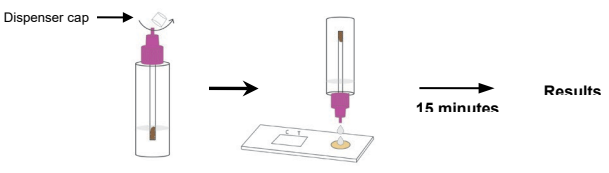
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

Step 2: When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.

Step 3: Shake the stool collection device vigorously to ensure a homogenous liquid suspension.

Step 4: Position the stool collection device upright and twist off the dispenser cap.

Holding the stool collection device vertically, dispense 2 drops of the solution (85- 95 µL) into the sample well of the test device. Do not overload specimen.



Step 5: Set up timer.

Step 6: Results can be read at 15 minutes. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of 20 minutes only. ***Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

QUALITY CONTROL

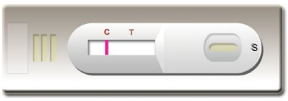
1. Internal Control: This test contains a built-in control feature, the C line. The C line

develops after adding specimen. Otherwise, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that the level of rotavirus antigen in the specimen is undetectable. The result is rotavirus Ag negative or non-reactive.



2. **POSITIVE RESULT:** If both the C line and the T line develop, the test indicates the presence of rotavirus antigen. The result is rotavirus Ag positive or reactive.

Specimens with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic decision is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

107 fecal samples were collected from subjects with symptomatic diarrhea or non-diarrheal symptoms and tested with the *OnSite* Rotavirus Ag Rapid Test and with a reference rotavirus antigen rapid test. Comparison for all subjects is shown in the following table:

	OnSite Rotavirus Ag Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	36	0	36
Negative	2	69	71
Total	38	69	107

Relative Sensitivity: 100% (95% CI: 90.3-100%),
Relative Specificity: 97.2% (95% CI: 90.2-99.7%),
Overall Agreement: 98.1% (95% CI: 93.4-99.8%).

2. **Serotype Detection**

The *OnSite* Rotavirus Ag Rapid Test detects Group A rotavirus.

3. **Cross-Reactivity**

The cross-reactivity of the *OnSite* Rotavirus Ag Rapid Test was assessed by testing fecal specimens collected from patients with other gastro-intestinal infectious diseases.

Fecal Specimens	Sample Size	Rotavirus Ag Reactivity
Typhoid fever	6	Negative
Adenovirus	10	Negative
H. pylori	10	Negative
Cholera (spiked)	3	Negative

LIMITATIONS OF THE TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of rotavirus Ag in feces. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Rotavirus Ag Rapid Test is limited to the qualitative detection of rotavirus Ag in human fecal specimen. The intensity of the test line does not have linear correlation with antigen concentration in the specimen.
3. A negative result for an individual subject indicates absence of detectable rotavirus antigen. However, a negative test result does not preclude the possibility of infection with rotavirus.
4. A negative result can occur if the quantity of the rotavirus antigen present in the specimen is below the detection limits of the assay or the antigens that are detected are not present in the fecal sample collected.
5. Infection may progress rapidly. If the symptoms persist and the result from the *OnSite* Rotavirus Ag Rapid Test is negative or non-reactive, it is recommended to test with alternative test methods.
6. The use of meconium stools in this assay is not recommended, as their performance characteristics have not been evaluated.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number		Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

PI-R0194C Rev. A2.1
Date released: 2020-11-12
English version

For Export Only, Not For Resale in the USA

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany