OnSite™ Rota/Adeno Ag Rapid Test

REF R0196C CE

Instructions for Use

INTENDED USE

The *OnSite* Rota/Adeno Ag Rapid Test is a lateral flow immunoassay for the qualitative detection and differentiation of rotavirus and adenovirus antigens in fecal specimens. It is intended to be used by healthcare professionals to aid in the diagnosis of infection with rotavirus and adenovirus.

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 the third leading cause of death related to infectious diseases throughout the world. The rate of death due to diarrheal diseases is estimated as 1.7-2.5 million per year[1]. A number of bacterial, parasitic, and viral pathogens have been identified as causes of acute diarrheal gastroenteritis. Rotaviruses, which are adenoviruses, account for large percentage of reported cases[2-5].

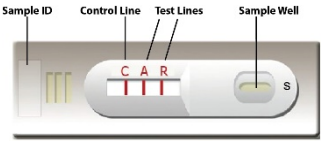
Rotavirus A is the most common cause of viral gastroenteritis in children under 5 years of age and results in approximately 500,000 deaths annually with the majority occurring in the developing world[6]. Rotavirus infection is more frequently observed in winter months in temperate climate conditions, but has less distinct seasonality in tropical climates[7,8]. Generally, the clinical manifestations of rotavirus infections are more severe than other viral infections. Symptoms include the sudden onset of fever with severe diarrhea and vomiting, which can lead to dehydration. Vomiting lasts for 2-3 days and diarrhea is observed for 4-5 days on average[6,9].

Adenovirus type 40 and type 41 account for up to 20% of viral gastroenteritis in young children globally, primarily affecting pediatric patients less than 2 years old[4,5,10]. Adenoviruses do not demonstrate the seasonal distribution pattern[5,6] observed in rotavirus infection[11]. Clinical characteristics include watery diarrhea accompanied by vomiting and low-grade fever. High fever and dehydration are less frequently observed in comparison to rotavirus infections. A distinct feature of adenovirus infections is the protracted diarrhea and longer duration of symptoms[12].

Diagnosis of rotavirus and adenovirus gastroenteritis is important towards decreasing the unnecessary use of antibiotics, especially in the outpatient clinics with high patient volumes. Specific diagnosis of infection with rotavirus and adenovirus through the detection of virus antigen in stool by immunoassay methods is widely used in clinical settings[13,14].

The *OnSite* Rota/Adeno Ag Rapid Test qualitatively detects and differentiates rotavirus antigen and adenovirus antigen in fecal specimens. The test can be performed within 15-20 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Rota/Adeno Ag Rapid Test is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing monoclonal anti-rotavirus antibody conjugated with colloidal gold (anti-rotavirus conjugates) and monoclonal anti-adenovirus antibody conjugated with colloidal gold (anti-adenovirus conjugates) and 2) a nitrocellulose membrane strip containing two test lines (R line and A line) and a control line (C line). The R line is pre-coated with anti-rotavirus antibody, the A line is pre-coated with anti-adenovirus antibody, and the C line is pre-coated with a control line antibody.

When an adequate volume of test 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 anti-rotavirus antibody forming a colored R line, indicating a rotavirus positive test result.

Adenovirus Ag, if present in the specimen, will bind to the anti-adenovirus conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-adenovirus antibody forming a colored A line, indicating an adenovirus positive test result.

Absence of the test lines suggests a 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 any of the test lines. 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-R0196)
3. Plastic droppers for transfer of 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 from 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. Follow the US CDC Universal Precautions for bio-safety.
10. **Do not scoop fecal specimen as this may lead to excess fecal specimen that tends to clot the sample pad and interfere with sample migration.**
11. Read the test results 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 unused test devices and stool collection devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test devices and stool collection devices containing sample extraction buffer are brought to room temperature before opening. The test device is stable until the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

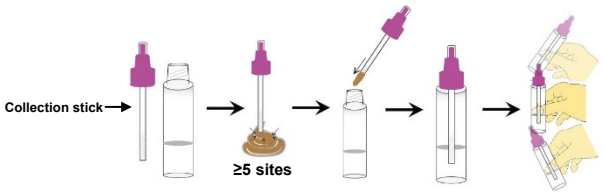
SPECIMEN COLLECTION AND HANDLING

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.

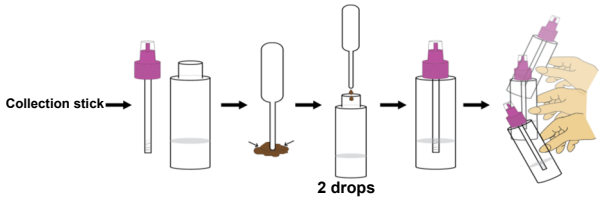
Procedure A: Solid stool samples

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. Ensure that stool specimen is only in the grooves of the collection stick. Excess stool specimen may lead to an invalid test result.***
Step 3: Replace the collection stick and tighten securely to close the stool collection device.
Step 4: **Shake the stool collection device vigorously.**



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.

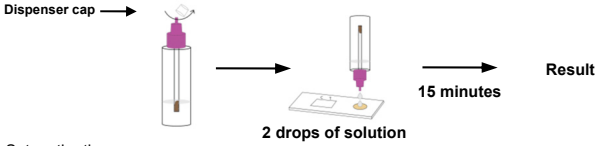
ASSAY PROCEDURE

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: Hold the stool collection device vertically. Twist off the cap. dispense 2 drops (85-95 µL) of the solution into the sample well of the test device. Do not overload specimen.



Step 5: Set up the 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. If the C line does not develop, review the whole procedure and repeat the test using a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure 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 kit 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 no detectable rotavirus antigen and adenovirus antigen is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT:**

2.1 In addition to the presence of the C line, if the R line develops, the test indicates the presence of rotavirus antigen. The result is rotavirus Ag positive or reactive.

2.2 In addition to the presence of the C line, if the A line develops, the test indicates the presence of adenovirus antigen. The result is adenovirus Ag positive or reactive.

2.3 In addition to the presence of the C line, if both the R line and the A line develop, the result indicates the presence of both rotavirus antigen and adenovirus antigen. The result is both rotavirus Ag and adenovirus Ag positive or reactive.

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

3. **INVALID RESULT:** If no C line develops, the assay is invalid regardless of any color development in the R line or A line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance of Rotavirus Ag Test**

A total of 107 fecal samples were collected from subjects with or without symptomatic diarrhea and tested with the *OnSite* Rota/Adeno Ag Rapid Test and with a reference Rota/Adeno Ag rapid test. Comparison for all subjects is shown in the following table:

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

Relative Sensitivity: 100%, Relative Specificity: 97.2%, Overall Agreement: 98.1%

2. **Clinical Performance of Adenovirus Ag Test**

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

	***OnSite* Rota/Adeno Ag Rapid Test**		
Reference	**Positive**	**Negative**	**Total**
Positive	10	0	10
Negative	2	95	97
Total	12	95	107

Relative Sensitivity: 100%, Relative Specificity: 97.9%, Overall Agreement: 98.1%

3. **Cross-Reactivity**

No false positive rotavirus Ag or adenovirus Ag test results were observed on 3-15 specimens from the following disease states:

Fecal specimens	**Sample size**	**Rotavirus Ag Reactivity**	**Adenovirus Ag Reactivity**
Typhoid fever	6	Negative	Negative
Rotavirus	15	Positive	Negative
Adenovirus	10	Negative	Positive
H. pylori	10	Negative	Negative
Cholera (spiked)	3	Negative	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 or adenovirus Ag in feces. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Rota/Adeno Ag Rapid Test is limited to the qualitative detection of rotavirus antigen and adenovirus antigen in human fecal specimens. The intensity of the test line does not have a linear correlation with antigen concentration in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable rotavirus antigen or adenovirus antigen. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with rotavirus or adenovirus.
4. A negative or non-reactive result can occur if the quantity of the rotavirus antigen or adenovirus antigen present in the specimen is below the limits of detection or if the antigens that are detected are not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptoms persist, while the result from *OnSite* Rota/Adeno 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

1. Diarrheal Diseases (updated February 2009) http:// www.who.int/vaccine_research/diseases/ diarrhoeal/en/index4.html
2. Parashar, U, Gibson CJ, Bresee JS, et al. Rotavirus and severe childhood diarrhea. Emerg Infect Dis (2005) 12:304-306.
3. Parashar UD, Bresee JS, Gentsch JR, et al., Rotavirus. Emerg Infect Dis (1998) 4(4):561-570.
4. Shinozaki, T., et al. Epidemiology of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children in the Tokyo area. Scand J Infect Dis (1991) 23:543-547.
5. Uhnoo, I, Wadell, G, Svensson, L, et al. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. J Clin Microbiol (1984) 20:365-372.
6. Parashar UD, Hummelman EG, Breese JS, et al. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003, 9(5):565-572.
7. Levy, K, Hubbard, AE and Eisenberg, JN. Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. Int J Epidemiol (2009) 38:1487-1496.
8. Cook, SM, Glass, RI, et al. Global seasonality of rotavirus infections. Bull WHO. (1990) 68:171-177.
9. Farkas T and Jiang XI. Rotaviruses, Caliciviruses, Astroviruses, Enteric adenoviruses and Other Diarrheic Viruses. In *Manual of Clinical Micriobiology* 9th edition. Edited by: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. Washington DC:ASM Press; 2007:1453-69.
10. Brandt, CD, Kim HW, Rodriguez WJ et al. Adenoviruses and pediatric gastroenteritis. J Infect Dis (1985) 151:437-443.
11. Dey, R.S., Ghosh, S., Chawla-Sarkar, M., et al. Circulation of a Novel Pattern of Infections by Enteric Adenovirus Serotype 41 among Children below 5 Years of Age in Kolkata, India. J Clin Microbiol (2011) 49:500-505.
12. David O: Matson. Rotavirus, Enteric adenoviruses, Caliciviruses, Astroviruses and other viruses causing gastroenteritis. In *Clinical Virology Manual* 3rd edition. Edited by: Steven Specter, Richard L Hodinka, Stephen A Young. ASM Pres; 2000:275-77.
13. Dennehy, P.H., D.R. Gauntlett, and S.E. Spangenberger. Choice of reference assay for the detection of rotavirus in fecal specimens: electron Microscopy versus enzyme immunoassay. J Clin Microbiol (1990) 6:1280-1283.
14. Lipson, S.M., and K.A. Zelinsky. Comparison of four latex agglutination (LA) and three enzyme-linked immunosorbant assays (ELISA) for the detection of rotavirus in fecal specimens. Am J Clin Path (1989) 92: 637-643.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	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

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

PI-R0196C Rev. AP1.0
Date released: 2020-02-28
English version

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