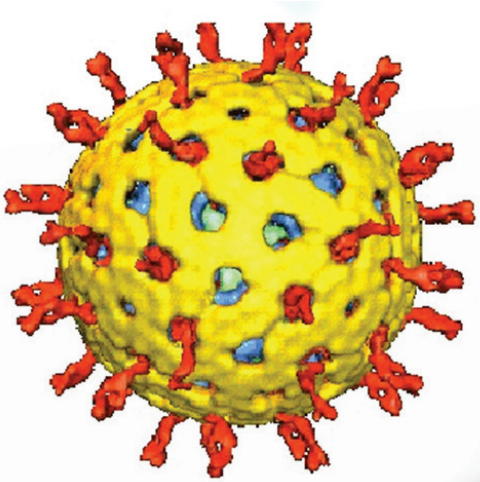
Rapydtest®

FOR THE DETECTION OF ROTAVIRUS/ADENOVIRUS IN FAECES



VIROLOGY

SINGLE USE IN VITRO DIAGNOSTIC DEVICE

APACOR

Rapydtest®

Rota/Adeno Ag

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Intended Use

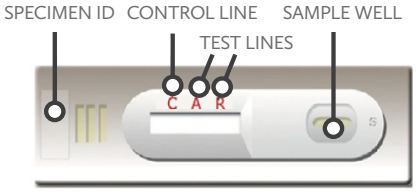
The Apacor Rota/Adeno Ag Rapydtest® is a lateral flow immunoassay for the qualitative detection and differentiation of rotavirus and adenovirus antigens in faecal specimens. This device is intended to be used as a screening test and as an aid in the diagnosis of infection with rotavirus and adenovirus.

Explanation of the Test

Diarrhoea is the third leading cause of death related to infectious diseases throughout the world. The rate of death due to diarrhoeal diseases is estimated as 1.7 - 2.5 million a year[1]. A number of bacterial, parasitic, and viral pathogens have been identified as causes of acute diarrhoeal gastroenteritis; rotaviruses and adenoviruses account for large percentages of 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 under 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 diarrhoea and vomiting which can lead to dehydration. Vomiting lasts for 2-3 days and diarrhoea 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 paediatric 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 diarrhoea 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 diarrhoea 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 Apacor Rota/Adeno Ag Rapydtest® utilises pairs of specific antibodies to qualitatively detect and differentiate rotavirus antigen and adenovirus antigen in faecal specimens. The test can be performed without cumbersome laboratory equipment, and the results are available within 15 minutes.

Principle

The Apacor Rota/Adeno Ag Rapydtest® is a lateral flow chromatographic immunoassay. The test strip consists of:



1. A burgundy coloured 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).
2. A nitrocellulose membrane strip containing two test bands (R band and A band) and a control band (C band). The R band is pre-coated with mouse anti-rotavirus antibody, the A band is pre-coated with mouse anti-adenovirus antibody, and the C band 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 burgundy coloured R band, 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 burgundy coloured A band, indicating an adenovirus positive test result. Absence of the test bands suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy coloured band of the immunocomplex of the control antibodies, regardless of colour development on any of the test bands. 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 2ml extraction buffer
3. Plastic droppers for transferring watery stool
4. One package insert (instruction 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. This information sheet must be read completely before performing the test. Failure to follow the information sheet can give 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°) 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 'Good Laboratory Practice' for biosafety.
10. **Do not scoop faecal specimen as this may lead to excess faecal specimen that tends to clot the sample pad and interfere with sample migration.**
11. Extraction buffer contains <0.1%NaN_3. Avoid contact with skin or eyes. Do not ingest.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well of the device. Reading results after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air conditioning.

Reagent Preparation and Storage Instructions

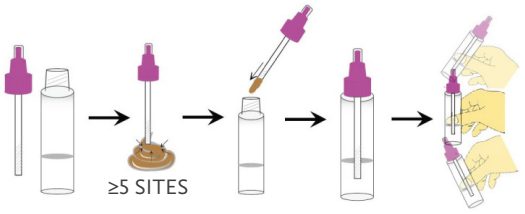
All reagents are ready to use as supplied. Store unopened test devices at 2-30°C. The positive and negative controls should be kept at 2-8°C or the temperature indicated. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the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

Specimen Collection and Handling

Consider any materials of human origin as infectious and handle them using standard biosafety procedures. To prepare specimens using solid stool samples follow Procedure A below. To prepare specimens using watery stool samples follow Procedure B below.

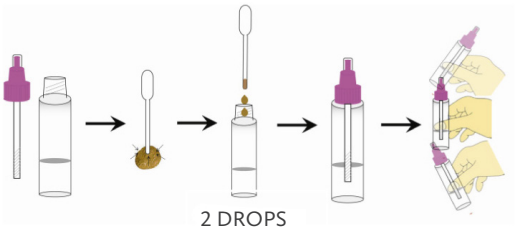
Procedure A: Solid stool samples

1. Collect a random sample of faeces in a clean, dry receptacle.
2. 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.*
3. Ensure stool sample is only in the grooves of the collection stick. *Excess stool sample may lead to an invalid test result.*
4. Replace the collection stick and tighten securely to close the stool collection device..
5. *Shake the stool collection device vigorously.*



Procedure B: Watery stool samples

1. Collect a random sample of faeces in a clean, dry receptacle.
2. Open the stool collection device by unscrewing the top.
3. Fill the plastic dropper with the sample; dispense 2 drops (70-85µl) into the stool collection device.
4. Replace the collection stick and tighten securely to close the stool collection device.
5. *Shake the stool collection device vigorously.*



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

Note: Specimens extracted may be stored at 2-8°C for up to 3 days. If longer storage is required, freezing at ≤-20°C is recommended.

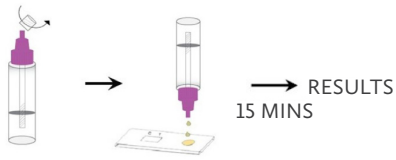
Test Procedure

STEP 1: Bring the specimen and test components to room temperature if refrigerated or frozen.

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 sample collection tube vigorously to ensure an effective 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 (85-95µl) of the solution into the sample well of the test device. Do not overload sample.



STEP 5: Set up timer.

STEP 6: Results can be read in 15 minutes. Positive or reactive results can be visible in a time period as short as 1 minute.

Do not read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

Quality Control

1. **Internal Control:** This test contains a built-in control feature, the C band. The C band develops after adding specimen. If the C band does not develop, 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** - 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 used 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 band is developed, the test indicates that the level of rotavirus Ag and adenovirus Ag in the specimen is undetectable. The result is negative.

2. **Positive Result**:

2.1 In addition to the presence of the C band, if the R band is developed, the test indicates that the specimen contains rotavirus Ag. The result is rotavirus Ag positive.

2.2 In addition to the presence of the C band, if the A band is developed, the test indicates that the specimen contains adenovirus Ag. The result is adenovirus Ag positive.

2.3 In addition to the presence of the C band, if both the R band and the A band are developed, the result indicates the specimen contains both rotavirus Ag and adenovirus Ag. The result is both rotavirus Ag and adenovirus Ag positive.

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Interpretation of Assay Result

3. **Invalid Result**: If no C band is developed, the assay is invalid regardless of any colour development in the R band or A band as indicated below. Repeat the assay with a new device.

Performance Characteristics

1. **Clinical Performance of rotavirus specimens:** 107 faecal samples collected from subjects with symptomatic diarrhoea and non- diarrhoea symptoms were tested with the Apacor Rota/Adeno Ag Rapydtest® and with a reference Rota/Adeno Ag rapid test. Comparison for all subjects is shown in the following table:

	APACOR ROTA/ADENO Ag RAPYDTEST®		
REFERENCE TEST	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 specimens:** 107 faecal samples collected from subjects with symptomatic diarrhoea and non-diarrhoea symptoms were tested with the Apacor Rota/Adeno Ag Rapydtest® and with a reference rapid test. Comparison for all subjects is shown in the following table:

	APACOR ROTA/ADENO Ag RAPYDTEST®		
REFERENCE TEST	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:** The cross reactivity of the Apacor Rota/Adeno Ag Rapydtest® was assessed by testing faecal specimens from patients with other gastrointestinal infectious diseases. As shown in the following table, no cross-reactivity was observed.

FAECAL 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 Test Procedure and the Interpretation of Test Result sections must be followed closely when testing for the presence of rotavirus Ag or adenovirus Ag in faeces. Failure to follow the procedure may give inaccurate results.
2. The Apacor Rota/Adeno Ag Rapydtest® is limited to the qualitative detection of rotavirus Ag and adenovirus Ag in human faecal specimens. The intensity of the test band does not have linear correlation with antigen concentration in the specimen.
3. A non-reactive result for an individual subject indicates absence of detectable rotavirus antigen or adenovirus antigen. However, a non-reactive test result does not preclude the possibility of exposure to or infection with rotavirus or adenovirus.
4. A non-reactive result can occur if the quantity of the rotavirus antigen or adenovirus antigen present in the specimen is below the detection limits of the assay or the antigens that are detected are not present during the stage of disease in which a sample is collected.
5. If the symptoms persist while the result from the Apacor Rota/Adeno Ag Rapydtest® is negative or non-reactive, it is recommended to re-sample the patient a few days later or 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.

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Ordering Information

PRODUCT	PACK SIZE	CODE
Rota/Adeno Ag Rapydtest®	25	1640

Products can be ordered direct from Apacor or from an appointed distributor

Visit our website for all the latest information www.apacor.com or e-mail on: orders@apacor.com

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