ADENO+ROTA CARD PLUS

For *in Vitro* diagnostic use only

Immunochromatographic test for the detection of Adenovirus and Rotavirus antigens in human faeces

I. INTRODUCTION AND INTENDED USE

Viral gastroenteritis is an infection caused by a variety of viruses that results in vomiting or diarrhea. Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, astroviruses and noroviruses.
The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 5-8 days. Rotavirus is the more frequent cause of acute diarrhea in children under two years of age. Adenoviruses and astroviruses cause diarrhea mostly in young children, but older children and adults can also be affected.
The Adeno+Rota Card Plus is a qualitative lateral flow immunoassay for the detection of Adenovirus and Rotavirus antigen in human faeces samples; the kit contain positive and negative controls.

II. PRINCIPLE

The membrane is pre-coated with monoclonal antibodies against *Rotavirus* and *Adenovirus* antigens on the test lines region. During testing, the sample reacts with the particle coated with anti-*Rotavirus* antibodies and/or with anti-*Adenovirus* antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugates and generate coloured lines red for Adenovirus and blue for Rotavirus). A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains:
1. Card for immunochromatographic reaction, (25).
2. Extraction buffer (25): 25 vial containing 1 mL of extraction solution.
3. Positive Control: N.1 vial with dropper containing non-infectious components, sodium azide (NaN_3) as preservative (1 x 0.5 mL).
Negative Control: use Extraction buffer-Diluent
4. Plastic droppers (6)
5. Instruction for use (1)

Required materials (not supplied)

Specimen collection container, Disposable gloves, Plastic pipettes, Timer or clock.

IV. PRECAUTIONS

- For *in* vitro and professional use only
- Do not use after expiration date.
- Stool specimen can be potentially infectious. Safety measures for handling as well as storing the collected specimen must be fixed by the operators.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

V. STORAGE

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SAMPLES AND PREPARATION

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing. Specimens may be frozen and thawed twice.

Liquid or Semi-Solid Stools

Using a separate pipette for each stool, draw stool of the sample itself. Dispense 6-7 drops of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds.
Care should be taken when pipetting semi-solid stool. The addition of less than indicated of stool may cause a false-negative test. The addition of more than indicated of stool may cause invalid results due to restricted sample flow.

Formed / Solid Stools

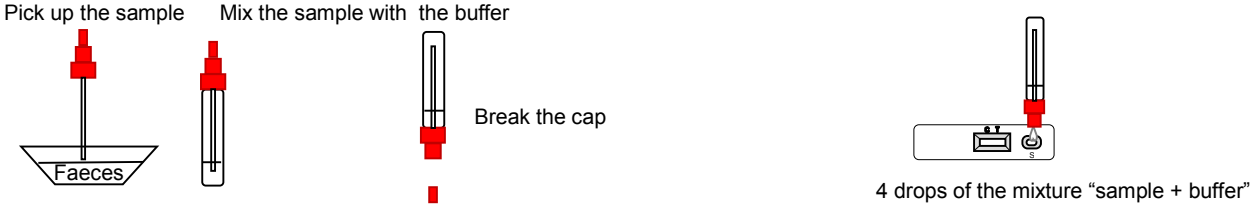
Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen. Verify to transfer a small portion (approximately 6 mm diameter) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Wait at least 3 minutes. Repeat the operations just to obtain a dark yellow-brown solution, if necessary. The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow.

VII. PROCEDURE

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

Procedure for the samples

1. Let all the reagents warm up to room temperature before proceeding with the test.
2. Stir thoroughly to homogenize the solution the vial with sample and extraction buffer.
3. Break the dropper of the vial and dispense 3-4 drops (150 µL) into the card's well.
4. Let react for 10 minutes. Results must be read on wet strip after 10 minutes incubation.
5. Interpret the results in accordance following schema.



Procedure for the controls: For the Positive and Negative Controls use the same procedure (from step 3 onwards: dispense 3-4 drops….)

X. INTERPRETING THE RESULTS

Interpreting the results for samples

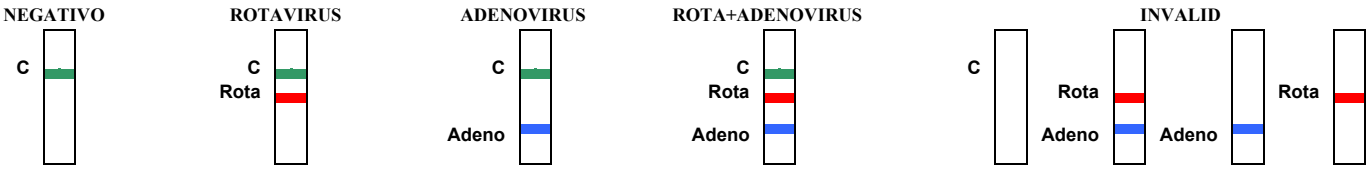
Negative test: only one line appears in the control line region (green control line).

Rotavirus Positive test: two lines appears: in the Result Line Region (red test line) and in the control line region (green control line).

Adenovirus Positive test: two lines appears: in the Result Line Region (blue test line) and in the control line region (green control line).

Rotavirus+Adenovirus Positive test: three lines appears: in the Result Line Region (red and blue test line) and in the control line region (green control line).

Invalid test: The absence of the migration control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.



Interpreting the results for samples

Negative control: only one line appears in the control line region (green control line).

Positive control: three lines appears: in the Result Line Region (two purple / pink test line) and in the control line region (green control line).

VIII. PERFORMANCE CHARACTERISTICS

An evaluation was conduced comparing the results obtained using ADENO+ROTA CARD PLUS (Mascia Brunelli) to commercial available ELISA assays.

ROTA (Mascia Brunelli)	ELISA assay* +	-	Total
+	18	1	19
-	0	43	43
Total	18	44	62

Sensitivity:	90%
Specificity::	>99%
PPV	>99%
NPV:	>98%

*Rotavirus ELISA assay: **Ridascreen**®Rotavirus (r-Biopharm).

ADENO (Mascia Brunelli)	ELISA assay* +	-	Total
+	9	2	11
-	2	17	19
Total	11	19	30

Sensitivity:	90%
Specificity::	>99%
PPV	>99%
NPV:	>98%

*Adenovirus ELISA assay: **Ideia**®Adenovirus (Dako-Diagnostics).

Cross-reactivity:

- No interferences with: Enterovirus, Astrovirus, Escherichia coli, Campylobacter, Giardia lamblia e Human Hemoglobin.

IX. LIMITS OF THE TEST

- Adeno+Rota Card Plus is screening test for determination of presence of Adeno/Rotavirus in human faeces. A definitive clinical diagnosis should be made by the physician after all clinical and laboratory findings have been evaluated.
- A positive result does not rule the possibility that other pathogens may be present.
- 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 Adenovirus/Rotavirus infection.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

X. BIBLIOGRAPHIC REFERENCES (See Italian version)

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (25 tests)	**Ref. VC19025P**
Card	25 items
Dilution buffer Vial (1,0 mL)	25 items
Positive Control (0,5mL)	1 item
Plastic dropper	6 items
Instruction for use	1 item

EDMA (EDMS) CODE 1570909000 CE IVD