INSTRUCTIONS FOR USE

ADENO+ROTA CARD PLUS

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE SIMULTANEOUS QUALITATIVE DETECTION OF *ADENOVIRUS* AND *ROTAVIRUS* IN HUMAN STOOL SAMPLES, INCLUDING POSITIVE CONTROL

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Viral gastroenteritis is an infection caused by a variety of viruses that result in vomiting or diarrhea. Many different viruses can cause gastroenteritis, including rotaviruses, noroviruses, adenoviruses, sapoviruses, and astroviruses.
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 1 to 10 days, depending on which virus causes the illness. Some research studies have shown that the duration of the symptoms are approximately three to four 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 manual, rapid immunochromatographic test for the simultaneous qualitative detection of *Adenovirus* and *Rotavirus* antigen in human faeces samples. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of *Rotavirus* and/or *Adenovirus* infection. The kit include the positive control for *Adenovirus* and *Rotavirus*.

2 - PRINCIPLE OF THE METHOD

ADENO+ROTA CARD PLUS is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of *Adenovirus* and/or *Rotavirus* antigens in stool samples.
The strip consists of a nitrocellulose membrane pre-coated with monoclonal antibodies against *Adenovirus* on the first test line (T), in the results window, and against *Rotavirus* on the second test line (T) and with rabbit polyclonal antibodies, on the control line (C), against a specific protein.
The label/sample absorbent pad is sprayed with test label solution (monoclonal antibodies anti-*Adenovirus*) conjugated to blue polystyrene latex; (monoclonal antibodies anti-*Rotavirus*) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming coloured conjugate complexes.
If the sample is *Adenovirus* positive, the antigen of the diluted sample reacts with the blue-coloured conjugate complex (anti-*Adenovirus* monoclonal antibodies-blue polystyrene microspheres) which was previously pre-dried on the absorbent pad. If the sample is *Rotavirus* positive, the antigen of the diluted sample reacts with the red-coloured conjugate complex (anti-*Rotavirus* monoclonal antibodies-red polystyrene microspheres) which was previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-*Rotavirus* and/or anti-*Adenovirus* antibodies present on the membrane (test lines) capture the coloured conjugate and the blue and/or red line will be visible. These bands is used to interpret the result.
If the sample is negative, there is no *Rotavirus* and *Adenovirus* antigen presence and yet, the antigen may be present in a concentration lower than the detection limit value, for which the reaction will not take place with the blue and red-coloured conjugate complex. The anti-*Rotavirus* and anti-*Adenovirus* antibodies present on the membrane (test lines) will not capture the antigen-red/blue-coloured conjugate complexes (not formed), for which the blue and red lines will not appear. Whether the sample is positive or not the mixture continues to move across the membranes to the immobilized specific antibodies placed in the control lines. The anti-specific protein antibodies present on both membranes will capture control green-conjugate complex and both control lines will always appear. The presence of these green lines serve as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
ADENO+ROTA CARD PLUS	Immunochromatographic test	**VC194025P** (25 tests)	25 sealed in foil pouch containing the device, with dessicant. 25 plastic tubes with dropper tip containing the extraction liquid. To use also as negative control (25 x 1 mL). 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN_3 as preservative (1 x 0.5 mL) 6 plastic droppers. Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- ADENO+ROTA CARD PLUS is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- This product is not classified as dangerous according to current European legislation.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test device and each extraction buffer vial are for single use only.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use, and the test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- Wear gloves when handling the sample.
- Disposable gloves, extraction buffer, test tubes, and used devices in a propre biohazard container.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- If the device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case is

recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.

- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - Storage conditions and shelf life

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Do not freeze.

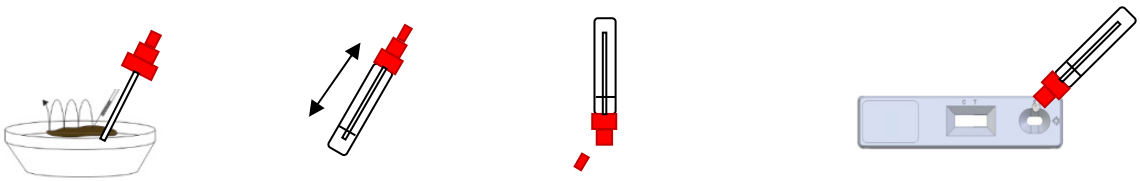
7 – Specimens and Preparation

Faecal samples: Stool samples should be collected in clean and dry containers. The samples can be stored in the refrigerator (2-8ºC) for **1-2 days** prior to testing. For longer storage, maximum **1 year**, the specimen must be kept frozen at -20ºC. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize stool sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended.

Samples preparation

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in *four* different places of the same stool specimen. Verify to transfer a small portion of stool (about 125 µL). Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. 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. For **liquid samples**, add approx. 125µL in the stool collection tube using a micropipette. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion.



8 - Test procedure

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

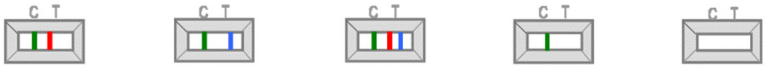
1. Remove the test card from the protective pouch. Identify the plastic cassette with the patients data.
2. Gently shake the test tube containing the sample under investigation. Brake the tip of the test tube.
3. Squeeze 4 drops of the extracted mixture into the sample well "S" of the card. Avoid adding solid particles with the liquid.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn´t work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

Procedure for controls: for positive and negative control (extraction buffer) use the same procedure for samples starting from point 3.

9 – Reading and interpretation

Interpret the results as follow:



Rotavirus Adenovirus Rotavirus-Adenovirus Negative Invalid

		Interpretation of results
1.	- RED - BLUE + GREEN	Absence of *Rotavirus* and *Adenovirus*. Negative result. No viral infection caused by *Rotavirus* and *Adenovirus.*
2.	+ RED + BLUE + GREEN	Presence of *Rotavirus* and *Adenovirus.* Viral infection caused by *Rotavirus* and *Adenovirus.*
3.	+ RED - BLUE + GREEN	Presence of *Rotavirus.* Viral infection from *Rotavirus*.
4.	- RED + BLUE + GREEN	Presence of *Adenovirus.* Viral infection from *Adenovirus*.
5.	- RED - BLUE - GREEN	Invalid results, we recommend repeating the assay using the same sample with another test.

INVALID: Total absence of any control coloured line (GREEN) regardless the appearance or not of the test lines (RED or BLUE). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red/blue coloured bands in the test line (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

10 - INTERNAL QUALITY CONTROL

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

11 – EXPECTED VALUES

Acute gastroenteritis is a common disorder in young children; moreover, the associated dehydration is a leading cause of admission to hospital in industrialized countries. Acute diarrhoea is a major health problem throughout the world and main source of mortality in developing countries. Enteric viruses have been recognized as the most significant etiological agents of the disease and yet, four categories of viruses are being considered clinically relevant: group A *Rotavirus* (family *Reoviridae*), *Norovirus* (family *Caliciviridae*), *Adenovirus* and *Astrovirus*. Several studies proved co-infections in infants 46% with acute watery diarrhoea.

12 - PERFORMANCES CHARACTERISTICS

Sensitivity and Specificity

An evaluation was conducted comparing the results obtained using the ADENO+ROTA CARD PLUS to a commercial available Rotavirus assay (Ridascreen®Rotavirus ELISA Test, r-Biopharm) and Adenovirus were confirmed using PCR.
ADENO-ROTA CARD PLUS was highly specific 98% to detect Rotavirus and >99% to detect Adenovirus and also highly sensitive >99% to detect Rotavirus and >99% to detect Adenovirus compared with the results of that membrane assay.

Cross reaction and Interferences

It was performed an evaluation to determine the cross reactivity of ADENO+ROTA CARD PLUS. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *Astrovirus, Campylobacter, C. difficile, Cryptosporidium parvum, Entamoeba histolytica, Enterovirus, E. coli, Giardia lamblia, H. pylori, Listeria monocytogenes, Norovirus, Salmonella, Shigella, Staphylococcus aureus.*

13 - LIMITATIONS OF THE METHOD

- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the extraction liquid and repeat the test.
- ADENO+ROTA CARD PLUS should only be used on human faecal samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
- Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
- A positive result determines the presence of *Adenovirus* and/or *Rotavirus* in the sample (qualitative determination). Neither a quantitative figure nor the rate of antigen increase can be determined with this test.
- A positive result must be followed by further laboratory techniques to confirm the results. However, confirmation of infection should only be made by the physician after evaluation of all clinical and laboratory findings and should be based on correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigens concentration in the stool sample is lower than the detection limit value. If the symptoms or situation still persist, additional testing using other clinical methods is recommended.

14 - REFERENCES

1. SILVA DE OLIVEIRA, CONSUELO; LINHARES, ALEXANDRE C. et al., “Rotavirus: clinical features and prevention”, Jornal de Pediatria - Vol. 75, Supl.1, 1999.
2. GUILLERMO BERNAOLA, WALTER LUQUE. et al., “Fisiopatología de las Infecciones por Adenovirus”, Paediatrica Asociación de Médicos Residentes del Instituto de Salud del Niño Oct. 2001 - Mar. 2002 Volumen 4, Nº 2 Págs. 41 - 47.

TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 4	Updated layout and content	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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