ROTAVIRUS CARD PLUS

For in *Vitro* diagnostic use only

Rapid test on format card for the detection of Rotavirus in human stool specimen, with positive and negative controls

I. INTENDED USE

Rotavirus Card Plus is a rapi immunochromatographic assay for qualitative detection of Rotavirus antigens in stool samples to aid in the diagnosis of Rotavirus infection.

II. PRINCIPLE OF THE TEST

The Rotavirus Card Plus Mascia Brunelli is a qualitative lateral flow immunoassay for the detection of *Rotavirus* antigen in human stool samples. The membrane is pre-coated with monoclonal antibodies against Rotavirus antigens on the test line region. During testing, the sample reacts with the particle coated with anti-Rota antibodies which was pre-dried on the test strip. The mixture moves 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 conjugate and generate a red coloured line in "T" area. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green coloured band always appears. The presence of this green band 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 all materials needed for 25 tests:
25 cards for the immunochromatographic reaction. Once opened, the test must be carried out within 2 hours.
25 vials containing 1,0 mL of Extraction solution.
6 Plastic droppers
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
1 Instruction for use
Store the kit at temperature between 2-30ºC. Do not freeze.
Required materials (not supplied)
Specimen collection container - Disposable gloves – Timer.

IV. PRECAUTIONS

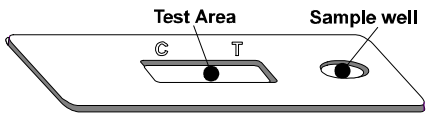
- For in vitro diagnosis only
- Read the instruction for use before performing the test
- Do not use after expiration date
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. It is suggest to disinfect or autoclave at 121°C for 1 hour
- Do not use the test if pouch is damaged

V. SPECIMENS COLLECTION

Stool specimen should be collected as soon as possible after onset of symptoms. Do not collect specimens in containers having media, preservatives, animal serum or detergents, as any of these may interfere with the test. The viral peak occurs 3-5 days after the onset of symptoms.
Diluted samples may be stored at +2°C to +8°C for 3 days without interference with assay performance. For long term storage of undiluted specimens, storage at –20°C or colder is recommended. Repeated freezing and thawing of samples is not recommended and may cause erroneous results.
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. 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.

VI. PROCEDURES FOR STOOL SAMPLES

1. Allow the reagents to reach to room temperature prior to testing. Remove the test card from the protective pouch. Identify with the patients data.
2. Gently shake the test tube containing the sample under investigation.
3. Brake the tip of the test tube and squeeze 5-6 drops (150 µL) of the extracted mixture into the sample well "S" of the card.
4. Read the result 10 minutes after the sample addition.



VII. PROCEDURE FOR CONTROLS

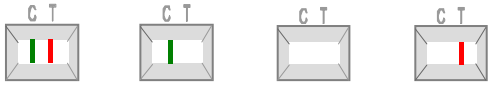
For the Positive and Negative Control use the same procedure (from step 3 onwards)

VIII. INTERPRETING THE RESULTS

Negative Test: Only one GREEN control band appears across the central window in the site marked with the letter C (control line). The sample is negative for Rotavirus.

Positive Test: In addition to the GREEN control band across the central window in the site marked with the letter C (control line), a red band (test line) also appears in the site marked with the letter T (result region). The sample is positive for Rotavirus.

Invalid: A total absence of the control coloured band. Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.



Positive Negative Invalid

IX. PERFORMANCE
SENSITIVITY and SPECIFICITY
An evaluation was conducted comparing the results obtained using the Rotavirus Card to a commercial available Rotavirus ELISA assay. Rotavirus Card was highly specific (>98%) and also highly sensitive (>99%) compared with the results of that ELISA assay.
CROSS-REACTIVITY
It was performed an evaluation to determine the cross reactivity of Rotavirus Card. There is not cross reactivity with common gastrointestinal pathogens, other organisms and substances occasionally present in faeces: Astrovirus, Adenovirus , Escherichia coli , Campylobacter, Giardia lamblia, Human Haemoglobin.

X. LIMITS OF THE KIT
- Rotavirus Card will only indicate the presence of Rotavirus in the specimen (qualitative detection) and should be used for the detection of Rotavirus antigens in faeces specimens only. To confirm the diagnosis additional testing using other clinical methods is recommended
- A positive result does not preclude the possibility of infections by other pathogens.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control line.
- This kit is intended for professional use in the laboratory and the test should be performed by qualified personnel with adequate training. Can not be used by the patient at home.

XI. REFERENCES
1. New Immunochromatographic Method for Rapid Detection of Rotaviruses in Stool Samples Compared with Standard Enzyme Immunoassay and Latex Agglutination Techniques. I. Wilhelmi, J. Colomina, D. Martín-Rodrigo E, Roman, A. Sánchez-Fauquier. European Journal of Clinical Microbiology & Infectious Diseases, October 2001, p. 741-743
2. The Clatterbridge Hospital Study: Comparison of One-Step Assays to the DAKO ELISA. Department of Microbiology, Clatterbridge Hospital, Wirral, England.
3. Rapid Detection of Rotaviruses – Are we underestimating infection in African infants? J Dewar1, M de Beer1, E Elliott2 D Semenya and A Steele. MRC Diarrhoeal Pathogens Research Unit, Medunsa, Pretoria, South Africa. Ampath Laboratories, Johannesburg, South Africa. 2004-08-19

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. VC194022P**
Card for immunochromatographic reaction	25 items
Vials with extraction solution	25 x 1,0 mL
Plastic droppers	6 items
Positive Control	1 x 0.5 mL
Instruction for use	1 item

EDMA (EDMS) Code 1570909000 CE IVD