immunocard STAT!®
Rota-Adeno-Noro2

A one-step immunochromatographic test for the differential detection of Rotavirus, Adenovirus and Norovirus genogroups I and II in human feces.

REF **750320** IVD

INTENDED USE

The Immunocard STAT! Rota-Adeno-Noro2 immunoassay is a rapid in vitro procedure for the qualitative detection, in separate bands, of Rotavirus, Adenovirus and Norovirus genogroup I (GI) and genogroup II (GII) antigens in human stool. The test can be used as an aid in the diagnosis of virus-associated gastroenteritis. A positive signal in any of the test bands is indicative of the presence of a Rotavirus, Adenovirus and/or Norovirus infection.

SUMMARY AND EXPLANATION OF THE TEST

Rotavirus [1, 2, 3]:

Rotavirus is a double stranded RNA virus belonging to the Reoviridae family. They are viruses with a low infective dose and their transmission mechanism is direct contact by one person to another by a fecal-oral route and, less frequently, through contaminated water and food. The Rotavirus is one of the main etiological agents of acute gastroenteritis in the whole world and main causal agent of severe dehydration in children between 6 months and 2 years, both in developing countries, where it shows a high mortality, as well as in developed countries. At the age of 5 years, most children
(> 95%) have suffered at least one episode of gastroenteritis caused by Rotavirus. Although the development of vaccines is helping to reduce the incidence, only some countries have managed to implement them in their national immunisation program. Rotavirus is classified into seven antigenic serogroups (A to G). Only Groups A, B and C infect humans, with Group A being the cause of almost all cases.

Adenovirus [1]:

*Adenovirus is the third leading cause of viral gastroenteritis in child*ren (10-15%); they can also cause respiratory diseases and depending on the serotype, diarrhoea, conjunctivitis, cystitis, and others. At least 47 Adenovirus serotypes have been identified and in all of them the hexon antigen is present. Serotypes 40 and 41 are associated with gastroenteritis. The main clinical symptom of gastroenteritis caused by Adenovirus is diarrhoea, for 9 to 12 days, also occurring with fever and vomiting.

Norovirus:

Norovirus is a type of single-stranded, positive-sense RNA virus belonging to the Caliciviridae family [4, 5, 6]. They are highly contagious and their main transmission routes are by person-person contact and by contaminated food / water. The virus usually causes large epidemics in closed communities (hospitals, homes for the elderly, schools, nurseries, restaurants, cruise ships, etc.), where once it has been introduced, infection propagates rapidly. Several studies demonstrate that Norovirus is the main cause of viral gastroenteritis at any age worldwide and responsible for almost 50% of gastroenteritis outbreaks [6]. Norovirus are grouped in five genogroups (GI to GV) and within each genogroup this virus is classified into genotypes. The majority of clinical cases are as a result of strains of the genogroups I and II being the genotypes GI.1 and GII.4 the most common ones [7, 8]. In general, GI infections are less frequent than GII infections [9, 10].

BIOLOGICAL PRINCIPLES

The Immunocard STAT! Rota-Adeno-Noro2 test contains two strips placed in a double cassette. The test is based on the immunological capture of coloured microparticles as they pass along a membrane on which specific monoclonal antibodies against Rotavirus, Adenovirus and Norovirus GI and GII have been immobilized in four separate bands.

Rota-Adeno strip uses a combination of:

1. Blue latex particles conjugated to a monoclonal antibody to Adenovirus hexon antigen and solid-phase polyclonal adenovirus antibodies. If Adenovirus is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-blue particle conjugate which can be seen visually as a blue line in the Adenovirus Test zone.
2. Red latex particles conjugated to a monoclonal antibody to VP6 antigen of the Rotavirus Group A and solid-phase polyclonal rotavirus antibodies. If Rotavirus is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-red particle conjugate which can be seen visually as a red line in the Rotavirus Test zone.
3. Green latex particles conjugated to an animal protein and the immobilized anti-protein on the membrane in the control zone. The Control line serves as a procedural control to assure that the sample has migrated the appropriate distance along the membrane to permit a valid test to be read.

Norovirus strip uses a combination of:

1. Red latex particles conjugated to monoclonal antibodies to GII and solid-phase polyclonal Norovirus GII antibodies.
2. Red latex particles conjugated to monoclonal antibodies to GI which corresponds with antibodies specific for GI.
3. Green latex particles conjugated to an animal protein and the immobilized anti-protein on the membrane in the Control zone. The Control line serves as a procedural control to ensure that the sample has migrated the appropriate distance along the membrane to permit a valid test to be read.

A diluted patient stool sample is dispensed into both sample ports of the cassette and migrates along the membranes through the Tests and Control zones. After 15 minutes of incubation at room temperature, the appearance of a specific coloured line in the reading window next to the corresponding letter indicates a positive result to specified virus in presence of a green Control line. (see Fig. 1 & 2).

REAGENTS/MATERIALS PROVIDED

The maximum number of tests obtained from this kit is listed on the outer box.

1. Immunocard STAT! Rota-Adeno-Noro2 device: Two reactive strips housed in a plastic frame and enclosed in a foil pouch with a desiccant.
2. Sample Diluent: Buffered solution containing 0.095% of sodium azide as preservative. The diluent is supplied in a red-capped plastic dropper vial with an applicator tip. Use as supplied.
3. Transfer pipettes

MATERIALS NOT PROVIDED

1. Positive and Negative Controls (Meridian Cat# **750301**)

2. Vortex
3. Interval Timer

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Do not deviate from the method described here or falsely positive or falsely negative results may occur. Once the assay has started, complete all subsequent steps without interruption.
3. Patient specimen and used Immunocard STAT! Rota-Adeno-Noro2 device may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories".
4. Do not interchange reagents from different kit lot numbers and do not use expired reagents.
5. Do not use the sample dilution buffer with evidence of contamination or precipitation.
6. The sample dilution buffer contains sodium azide which is a skin irritant. Avoid skin contact with reagents. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.
7. Dilution of stool as described under 'Specimen Collection and Preparation' is important. Over-inoculation of stool into the Sample Diluent may restrict movement within the Immunocard STAT! Rota-Adeno-Noro2 device so as to produce an invalid result.
8. In case the primary packaging is damaged (aluminum pouch or diluent buffer vial) the product should be discarded and not used.
9. Do not use this product if a colored line appears in the result area of any strip before you start to use it.

HAZARD AND PRECAUTIONARY STATEMENTS
There are no known hazards associated with this product.

SHELF LIFE AND STORAGE
Store the Immunocard STAT! Rota-Adeno-Noro2 kit at 2-30 C when not in use. The shelf life (expiry) for this product is listed on the kit box label.

PROCEDURAL NOTES
1. Allow kit components and specimens to reach room temperature (19-27 C) before performing a test, as cold reagents and/or specimens may decrease assay sensitivity. Reagents may take 30-40 minutes to warm up following refrigeration.
2. Stool samples must be mixed thoroughly (regardless of consistency) to ensure a representative sample prior to sampling.
3. Hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.
4. On occasion, particulate matter may interfere with sample flow. In cases where the Test Device does not readily absorb the diluted specimen, gently touch the bottom of the sample port with an applicator stick, moving the stool solid particle that might prevent the absorption. Alternatively, a new aliquot of the sample can be withdrawn from the Diluent and retested. Diluted samples containing a heavy concentration of particulate matter may be centrifuged
   (1-5 minutes at 700 x G) or allowed to stand for 3-5 minutes before proceeding.

REAGENT PREPARATION
Reagents are supplied ready to use. Allow kit components and specimens to reach room temperature (19-27 C) prior to use. Gently mix liquid reagent prior to use.

SPECIMEN COLLECTION AND PREPARATION
The faecal sample should be collected as soon as symptoms appear (especially diarrhoea and vomiting), as elimination of the virus in the faeces is maximal during the first three days after infection. Do not use stool in transport media, on swabs, or mixed with preservative or enrichment media.
The specimen should be transported in an airtight container and stored at 2-8 C on reception at the Laboratory, until tested. The specimen should be tested as soon as possible, but may be held up to 48 hours at 2-8 C prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (≤ -20 C). Specimens may be frozen and thawed once as multiple freeze-thaw cycles may alter the virus detection.
NOTE: Blood may cause instability problems especially when content in specimen is high. In this case, a visible change in the green color of the control band may occur.

Mix stool samples thoroughly to obtain an aliquot as representative as possible.

1. Liquid or semi-solid stool:
   Unscrew the red cap from the Sample Diluent vial (red capped vial). Transfer 4 drops of homogenized sample using the disposable pipette included in the kit or a volume of 110 µL. Use the same transfer pipette to mix the diluted sample by squeezing the pipette bulb three times. When the sample is semi-solid (unable to be pipetted), take a sample amount that completely covers the grooves of the stick attached to the vial cap. Recap the vial tightly and mix for 15 seconds using a vortex mixer.
2. Formed/Solid stool:
   Unscrew the red cap of the Sample Diluent vial (red capped vial). Use a wooden applicator stick or the white plastic applicator stick in the red cap to collect a small portion of stool
   (110 mg or 5-6 mm portion). Transfer the portion to the Sample Diluent vial. Recap the vial tightly and mix thoroughly for 15 seconds using a vortex mixer.

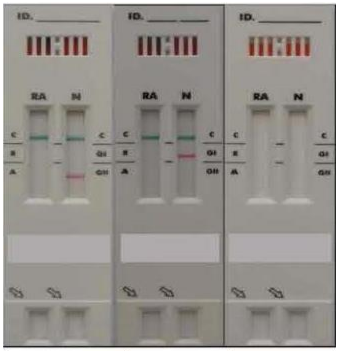
NOTE: The addition of less than 110 µL (110 mg) of stool may cause a false-negative test. The addition of more than 110 µL (110 mg) of stool may cause invalid results due to restricted sample flow.

TEST PROCEDURE
1. Bring all test devices, reagents and samples to room temperature (19-27 C) before testing.
2. Use one Immunocard STAT! Rota-Adeno-Noro2 Test Device for each patient sample. Remove the Immunocard STAT! Rota-Adeno-Noro2 Test Device from its foil pouch. Test Device is marked to indicate where tests and control lines will appear. The windows marked with an arrow are the test windows where sample is added.
3. Label the device with the patient's name.
4. Hold the diluted specimen vial upright and tap the bottom gently on the countertop before proceeding. Cover the top of the diluted sample vial with absorbent paper to avoid splatter. Break off the red tip on the outside of the red cap. (Do not break off the white applicator stick on the inside of the cap.)
5. Hold the vial upside down and dispense 4 drops of diluted sample into each rectangular window (marked with an arrow) of the Test Device. Do not touch the tip of the vial to the Test Device. Avoid adding solid particles of the sample.
6. Incubate the test at 19-27 C for 15 minutes and then read the results as described under INTERPRETATION OF RESULTS section below. Any line that may appear after 15 minutes does not have diagnostic value.

INTERPRETATION OF RESULTS
Test results are to be used in conjunction with information available from the patient via other diagnostic procedures.
Fig. 1 and 2 illustrate different results possible with this product, for each strip.

R/A - Noro -	Rota + Adeno - Noro -	Rota - Adeno + Noro -

Fig. 1: Patterns of possible results I



R/A - Noro GII +	R/A - Noro GI +	Invalid

Fig. 2: Patterns of possible results II

NEGATIVE RESULTS

Only a GREEN colored line appears at the Control line marked 'C' on both strips. These are the control bands and they should always appear as an indication that the chromatography has run smoothly in both strips.

POSITIVE RESULTS

Adenovirus positive

In the Rota-Adeno strip and in addition to the GREEN Control line, a BLUE line appears next to the letter "A". In the Norovirus strip, just a GREEN line appears at the Control line.

Rotavirus positive

In the Rota-Adeno strip and in addition to the GREEN Control line, a RED line appears next to the letter "R". In the Norovirus strip, just a GREEN line appears at the Control line.

Norovirus GII positive

In the Norovirus strip and in addition to the GREEN Control line, a RED line next to "GII" appears. In the Rota-Adeno strip, just a GREEN Control line appears.

Norovirus GI positive

In the Norovirus strip and in addition to the GREEN Control line, a RED line next to "GI" appears. In the Rota-Adeno strip, just a GREEN Control line appears.

INVALID RESULTS

1. No control bands appear,
2. or the colour of the control bands is completely different,
3. or band of any colour other than the defined colours appear in the reading zone.

NO REPORTABLE RESULT. Repeat test using the original sample to eliminate procedural potential error.

Obtain a new sample and retest when the original sample repeatedly produces unreadable results. In case of sample with high blood content, Meridian recommends using an alternative method as the instability issue may be linked to the specimen matrix itself.

LIMITATIONS OF THE PROCEDURE

1. The Immunocard STAT! Rota-Adeno-Noro2 is used for the differential identification of Rotavirus, Adenovirus and Norovirus GI and GII by detecting their presence in human stool samples if and when the viral load is the same or higher than the detection limit of the product for each analyte.
2. This is a qualitative test and no quantitative interpretation of the results should be made with respect to the intensity of the positive line.
3. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
4. Based on test evaluation on high numbers of stools, the Immunocard STAT! Rota-Adeno-Noro2 test shows a good correlation with other techniques (such us RT-PCR, ELISA and rapid tests). However, this study does not exclude possible interference in the performance of the test in other populations.
5. The Immunocard STAT! Rota-Adeno-Noro2 test has not been validated with all Norovirus genotypes and, as a result, may fail to detect Norovirus due to the enormous antigenic diversity of current strains.
6. A negative result does not exclude the possibility of infection by Rotavirus and/or Adenovirus and/or Norovirus. Non-detection of these viruses may be the result of factors such as: collecting the sample at an inappropriate stage of the disease (when very little virus is eliminated in the faeces), incorrect storage of the sample, inadequate sample transport, presence of a Norovirus genotype not detected by the strip.
7. A positive result does not exclude the presence of other pathogenic agents, including co-infection of any of these viruses with other microorganisms. In any case, co-infections can only be clarified by differential diagnosis.
8. It has been observed that faecal samples with a high blood content negatively interfere with the test. This issue is usually accompanied by an alteration in the control bands colour. (see figures under the "Interpretation of Results" section).
9. The test may lead to positive results in faeces from patients to whom the oral solution Rotateq vaccine has been administered until 15 days after the administration.

SPECIFIC PERFORMANCE CHARACTERISTICS

An independent Laboratory compared the performance of the Immunocard STAT! Rota-Adeno-Noro2 test in parallel to PCR, except for the Adenovirus for which a commercial EIA was used as Reference Method. Tested samples were as follows:

- 82 Negative samples for Rotavirus and Adenovirus
- 88 Negative samples for Norovirus GI and GII
- 8 Positive samples for Norovirus GI
- 20 Positive samples for Norovirus GII
- 20 Positive samples for Rotavirus
- 20 Positive samples for Adenovirus

Table 1 provides the performance data of the Immunocard STAT! Rota-Adeno-Noro2:

Table 1: Performance data of the Immunocard STAT! Rota-Adeno-Noro2

	SENSITIVITY	SPECIFICITY
Rotavirus	> 99.9%	98.8%
Adenovirus	> 99.9%	97.6%
Norovirus GI	87.5%	98.9%
Norovius GII	95.0%	96.6%

ANALYTICAL SENSITIVITY
The lower limit of detection for the Rota-Adeno strip is 31 ng/mL for both Rotavirus and Adenovirus antigens. The lower limit of detection for this Noro strip is 12.5 ng/mL for NoV GI.1 and is 1.5 ng/mL for NoV GII.4. The limits do not vary from liquid to solid stool samples.

REPRODUCIBILITY
The reproducibility of the Immunocard STAT! Rota-Adeno-Noro2 was determined using a single lot of this assay. Ten replicates of three concentrations were prepared for each analyte. Each concentration was documented respectively as PC ("positive control "), LPC ("low positive control") and NC ("negative control") and tested same day by the same operator. 100% repeatability was obtained with these three critical concentrations for each analyte indicating a high intra-assay precision of the test.
INTER-DAY PRECISION
The inter-day precision was measured by preparing serial dilutions (sensitivity curves) for each analyte. Testing was performed by the same operator on four different days with a concordance of 100% in results.
INTER-OPERATOR PRECISION
Inter-operator precision was determined using serial dilutions for each analyte tested in duplicate by five operators on the same day. Differences were observed but in no case exceeded 1 two-fold dilution. The differences were considered as acceptable for a qualitative immunochromatographic technique.

TESTS FOR INTERFERING SUBSTANCES
The following substances were found to have NO effect on results when present in stool at the indicated concentration:
Racecadotril (5% p/v), Cimetidine (10% p/v), Loperamide (5% p/v), Metronidazole (10% p/v), Omeprazole (3% p/v), Ampicillin (15% p/v), Ibuprofen (20% p/v), Acetylsalicylic acid (30% p/v), Edulcorant (5% p/v), Palmitic acid (40% p/v), Barium sulfate (5% p/v), Mucin (5% p/v).

CROSS-REACTIVITY STUDIES
Cross-reactivity was evaluated spiking Positive and Negative stools with listed micro-organisms. None yielded a positive result in negative stool or interfered with detection of a positive stool.

BACTERIA:
Aeromonas baumanii, Aeromonas hydrophila, Aeromonas caviae, Bacillus spp., Burkholderia cepacia, Campylobacter coli, Campylobacter jejuni, Citrobacter freundii, Clostridium perfringens, Clostridium difficile, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Escherichia coli O111, Escherichia Coli O127, Escherichia coli O26, Escherichia coli O55, Escherichia coli O157:H7, Hafnia alvei, Helicobacter pylori, Klebsiella pneumoniae, Lactobacillus casei BL23, Lactococcus lactis MC1363, Listeria monocitogenes, Morganella morganii, Plesiomonas shigelloides, Proteus mirabilis, Proteus vulgaris, Proteus penneri, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas stutzeri, Salmonella cholerasuis, Salmonella enteric serogroup B, Salmonella enteric serogroup D, Salmonella typhi, Serratia marcescens, Shigella flexnerii, Shigella sonneii, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus viridans, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica.
VIRUSES:
Astrovirus, Adenovirus, Enterovirus, Rotavirus strain Wa, Rotavirus, Sapovirus, virus Aichi.
FUNGI/PARASITES/OTHER:
Blastocystis hominis, Candida albicans, Candida parapsilosis, Candida tropicalis, Cryptosporidium parvum, Entamoeba histolytica, Entamoeba coli, Giardia lamblia.