ROTA-ADENO-NORO MonlabTest®
MO-076006 20 TESTS

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

CE IVD

For professional use *in vitro* diagnosis. Store at 2 – 30ºC.

CLINICAL MEANING

The Rota-Adeno-Noro MonlabTest chromatographic immunoassay is a procedure for the qualitative detection, in separate bands, of Rotavirus, Adenovirus and Norovirus genogroup I (GI) and genogroup II (GII) antigens in human faeces. A positive signal in either test bands provides a good indication that should draw the attention of the clinician to the possible presence of a Rotavirus, Adenovirus and/or Norovirus infection and contribute to the diagnosis of the patient.

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.

- **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 faecal-oral route and, less frequently, through contaminated water and food. The Rotavirus is one of the main aetiological 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, the majority of 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 causing factor of almost all cases.

- **Adenovirus**[1]:

Adenovirus is the third leading cause of viral gastroenteritis in children (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 very 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].

PRINCIPLE OF THE METHOD

The Rota-Adeno-Noro Monlabtest contains two strips placed in a double cassette:

1. Rota-Adeno strip uses a combination of:

a. Blue latex particles conjugated to a specific antibody against Adenovirus hexon antigen that cooperates with an antibody specific for Adenovirus located on the membrane under the Rotavirus band.

b. Red latex particles conjugated to a specific antibody against VP6 antigen of the Rotavirus Group A that cooperates with a Rotavirus specific antibody located on the membrane under the control band.

c. Green latex particles conjugated to an antigen recognized by a specific antibody for said antigen, bound to the membrane, giving rise to the so-called control band for the test.

2. Norovirus strip uses a combination of:

a. Red latex particles conjugated to specific antibodies against GII which cooperates with antibodies specific for GII located on the membrane under the GI band.

b. Red latex particles conjugated to specific antibodies against GI which cooperates with antibodies specific for GI located on the membrane under the control band.

c. Green latex particles conjugated to an antigen recognized by an antibody specific for this antigen bound to the membrane forming the so called test control band.

In this product the sample is first treated with the sample dilution buffer (included in the kit) to extract the viruses from the faecal matrix. After the extraction, it suffices to add a specific volume of the supernatant to both reactive strips and wait 15 minutes. When the extracted sample flows through the test membrane of both strips the coloured particles migrate. In the case of a positive sample, the specific antibodies present in the corresponding membrane capture the coloured particles. Lines of different colours will be visible, depending on the type of virus contained in the sample. These lines are used to interpret the result after 15 minutes of incubation at room temperature (see Fig. 1 y 2).

KIT CONTENTS	MATERIALS NOT INCLUDED IN THE KIT
- 20 cassettes - 20 vials with diluent (1,5ml) - Disposable plastic pipettes - Instructions for use	- Vortex - Chronometer

PRECAUTIONS

1. The patient samples (faeces) must be handled with care as they may contain infectious agents. Disposable gloves must be worn throughout the whole process.

2. The sample dilution buffer contains sodium azide as an antimicrobial agent. Avoid direct contact with skin and mucosa. Discard appropriately. Do not use the buffer if there are indications of contamination or precipitation.

3. Do not store or prepare food, eat, drink or smoke in the area where the reagents and samples are handled.

4. Once the task is finished, clean the work surfaces with soap and water and finish by disinfecting with a suitable solution. Lastly, remove gloves and wash hands with soap and water rubbing them well.

5. Do not exchange components from kits with different lot numbers.

6. If the test is stored refrigerated, let all the kit components and faecal samples reach room temperature, because cold reagents and/or samples can reduce test functionality. About 20-30 minutes are usually sufficient for reaching room temperature.

7. Use the reagents only *in vitro*.

8. Do not use kit components after the expiration dates.

9. In case the primary packaging is damaged (aluminum pouch or diluent buffer vial) the product should not be used even though none of the components have been damaged.

10. It is very important to add the correct volume of the extracted sample to the two sample application zones marked with an arrow in the plastic device. If lower than indicated, the chromatography may not occur due to not enough sample reaches the reaction areas; if there is too much, brown lines may appear instead of the normal colours (see Fig. 1 and 2).

11. The used product should be discarded in compliance with current legislation.

12. Do not use this product if a coloured line appears in the result area of any strip before you start to use it.

13. It is very important to take the appropriate amount of sample: about 110 mg for solid samples (a small portion of about 5 mm diameter). If the sample is semi-liquid (unable to take it with a pipette) take an amount capable of covering the grooves of the stick attached to the vial cap. Finally add 110 µl if the sample is liquid (4 drops if the disposable pipettes provided with the kit are used). These amounts are extracted into the sample dilution buffer supplied in the vials included in the kit.

An excess of sample in relation to the indicated previously may prevent the chromatography from running correctly; this is especially critical with solid samples since it is not easy to take the recommended amount of sample.

14. Do not discard the kit box until all the content has been used. This box contains essential information about the CE mark of the product and the batch number.

STORAGE AND STABILITY

The Rota-Adeno-Noro MonlabTest kit can be stored at temperatures between +2ºC and +30ºC. Its expiry date is printed on the aluminum pouches.

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Revision: December 2017

SAMPLES

This product is designed to analyze human faecal samples. 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 samples that have been collected in transport media, or those with added preservative agents (such as formalin, SAF, PVA or similar) or enrichment media, as their presence could interfere with correct performance of the test.

For best results, fresh, untreated samples are preferable. If they need to be kept for a certain time they can be stored in a refrigerator (+2ºC to +8ºC) for 1 or 2 days. For longer time periods they should be frozen to -20ºC, bearing in mind that some samples may lose their immunoreactivity after freezing.

Pay special attention when analysing haemorrhagic samples since they usually cause non-specificity problems when the blood content is high. An indication of this instability of the test is usually a change in the green colour of the control band.

If frozen, ensure the samples have fully thawed at room temperature prior to proceeding with their analysis. Avoid freeze-thaw cycles as this may alter immunological recognition of the virus.

PREPARATION OF THE FAECAL SAMPLES

General Note: all the necessary protections should be used throughout the test procedure due the handling of infectious samples. Once the work is concluded, do not forget to comply with the hygiene procedures detailed in point 4 of the "Precautions" section.

The protocol for faecal samples preparation is:

1. Homogenize previously the sample to get an aliquot as much representative as possible.

2. Unscrew the cap from the vial with caution in order not to spill the sample diluent buffer. With **solid samples**, take with the stick attached to the vial cap an approximate amount of **110 mg** of feces (a small **portion of 5 mm** in diameter). If the **sample is semi-liquid** (unable to take it with a pipette), take a sample amount so that it **completely covers the grooves of the stick attached to the vial cap**. With **liquid samples**, take a volume of **110 µl** (**4 drops** if the disposable pipettes included in the kit are used).

3. Carefully add the sample into the vial containing the dilution buffer. Screw the cap well and shake vigorously to ensure a homogeneous mixture.

PROCEDURE

1. Take the reaction device out of the aluminum pouch. Dispose of the desiccant sachet as its sole purpose is to protect the test from moisture.

2. Break the top of the vial cap.

3. Invert the vial and add **4 drops** in the sample application area of each strip (rectangular windows marked with an arrow). Try not to add solid particles with the sample.

4. Wait **15 minutes** before reading and interpreting the results.

READING AND INTERPRETATION

The six images that appear in Fig. 1 and 2 illustrate some different results that can be obtained with this product.

In each strip, different coloured bands can appear delimited by black horizontal lines printed in both sides of the cassette (see Fig. 1 and 2).

Fig. 1. Rota-Adeno strip:

a. Blue band: indicates the presence of Adenovirus in the sample.

b. Red band: indicates de presence of Rotavirus in the sample.

c. Green band: this is the control band and indicates that the test has worked correctly.

Fig. 2. Norovirus strip:

a. Lower red band: indicates de presence of Norovirus genogroup II in the sample.

b. Upper red band: indicates the presence of Norovirus genogroup I in the sample.

c. Green band: this is the control band and indicates that the test has worked correctly.

The green control bands in both strips should always appear. The additional presence of other band/s indicates the presence of Adenovirus and/or Rotavirus and/or Norovirus.

Strip 1	Strip 2	Strip 3
		
R/A - Noro -	Adeno + Noro -	Rota + Noro -

Fig. 1: Patterns of possible results I

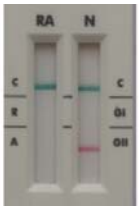
Strip 4	Strip 5	Strip 6
		
R/A - Noro GII +	R/A - Noro GI +	Invalid

Fig. 2: Patterns of possible results II

Strip 1: **NEGATIVE** result.

There is only a single horizontal **GREEN** line in both strips aligned with the letter "C" marked on both sides of the device. These are the control bands and they should always appear as an indication that the chromatography has run smoothly in both strips.

Strips 2-5: **POSITIVE** results

- Strip 2: **Adenovirus positive**

In the Rota-Adeno strip, a **GREEN** line aligned with the letter "C" and a **BLUE** line aligned with the letter "A" appear. In the Norovirus strip, just a **GREEN** line aligned with the letter "C" appears.

- Strip 3: **Rotavirus positive**

In the Rota-Adeno strip, a **GREEN** line aligned with the letter "C" and a **RED** line aligned with the letter "R" appear. In the Norovirus strip, just a **GREEN** line aligned with the letter "C" appears.

- Strip 4: **Norovirus GII positive**

In the Norovirus strip, a **GREEN** line aligned with the letter "C" and a **RED** line aligned with the symbol "GII" appear. In the Rota-Adeno strip, just a **GREEN** line aligned with the letter "C" appears.

- Strip 5: **Norovirus GI positive**

In the Norovirus strip, a **GREEN** line aligned with the letter "C" and a **RED** line aligned with the symbol "GI" appear. In the Rota-Adeno strip, just a **GREEN** line aligned with the letter "C" appears.

- Strip 6: **INVALID** results

No control bands appear, the green colour of the control bands is completely different, or non-specific and unexpected colours appear in the positive bands. This is an indication that the test has not worked correctly. This may be due to any of the following reasons:

- One or more of the reagents has deteriorated or the product has expired.
- The sample was not prepared according to the instructions for use.
- The sample has a high content of blood.

In the event of an invalid result, it is recommended that the test is repeated using a new cassette and to strictly adhere to the instructions for use described in this manual. In the case of samples with blood, it is advised to use an alternative technique as the instability problem does not usually depend on the strip used but on the matrix of the sample itself. Other commercial rapid tests gave similar results with these bloody samples.

Any line that may appear after 15 minutes will not have diagnostic value.

NOTE: the final and definite diagnosis should be made by a physician. This test only detects Rotavirus, Adenovirus and Norovirus in a sample. It does not constitute an argument to state that the person suffers from an infection due to these viruses.

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LIMITATIONS OF THE PROCEDURE

1. The Rota-Adeno-Noro test 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 as or higher than the detection limit of the product for each analyte.
2. This product is qualitative, not quantitative, although the intensity of the positive bands is related to the amount of virus detectable in the faecal sample.
3. The test shows a good correlation with other techniques (such us RT-PCR, ELISA and rapid tests) after analysing a high number of stools. However, this study does not exclude possible interference in the performance of the test when analysing other stool samples.
4. The Rota-Adeno-Noro test has not been validated with all the genotypes of Norovirus and, as a result, may fail to detect Norovirus due to the enormous antigenic diversity of current strains.
5. A defective sample may lead to very weak positive results. In the event of this occurring, the test must be repeated with a larger quantity of sample. On the other hand, excessive amount of sample may cause the test to develop very slowly and even prevent the test from developing correctly (the control bands are not visible). In the event of this occurring, the test must be repeated with a smaller quantity of sample (see section "Stool sample preparation").
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 the 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 high blood content negatively interfere with the test, with possible nonspecificity problems on samples that are negative for Rotavirus, Adenovirus and Norovirus. This instability of the test is usually accompanied by an alteration in the colour of the control bands (see the images in the "Reading the Results" section).
9. The results of the test should be interpreted together with any information available on epidemiological studies, clinical assessment of the patient and other diagnostic procedures.
10. 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.

SENSITIVITY AND SPECIFICITY

- **Rota-Adeno strip**: the analytical sensitivity is usually around 31 ng/ml both for Rotavirus and Adenovirus, although lower concentrations are usually detected.
- **Norovirus strip**: the MonlabTest internal control to validate the manufactured lots is a mixture of "virus like particles" GI.1+GII.4 as they are the most representative and common genotypes within each genogroup. The analytical sensitivity for NoV GI.1 and NoV GII.4 is 12.5 ng/ml and 1.5 ng/ml, respectively, although lower concentrations are usually detected.

It is important to note that the detection limit of the test was also analyzed using real stool samples. Values were consistent with those obtained from internal standards. These results demonstrate the robustness of the test.

DIAGNOSTIC SENSITIVITY AND SPECIFICITY

The Rota-Adeno-Noro MonlabTest was assessed against the following samples:

- 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.

The reference technique was PCR except for Adenovirus samples where a commercial rapid test was used.

The obtained results are indicated in the following table:

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

REPEATABILITY

Ten replicates of the three concentrations established as PC ("positivecontrol"), LPC ("low positive control") and NC ("negative control") for each analyte detected by this test were measured the same day by the same person. 100% repeatability was obtained with these three critical concentrations for each analyte indicating a high intra-assay precision of the test.

REPRODUCIBILITY

INTER-DAY PRECISION: Using a single lot of Rota-Adeno-Noro test, a sensitivity curve was measured for each analyte through four days spaced in time. The results were very reproducible (the same sensitivity for Rotavirus, Adenovirus, Norovirus GI and Norovirus GII through the four days of measurement).

INTER-OPERATOR PRECISION: Five operators measured in duplicate a sensitivity curve for each analyte. Differences were observed but in no case exceeded 1 two-fold dilution.

NOTE: The differences found in the different "Reproducibility" sections are acceptable for a qualitative immunochromatographic technique with its inherent variability.

HOOK EFFECT

Very high concentrations of the four analytes detected by the Rota-Adeno-Noro test were tested without observing any decrease in the intensity of the positive signals. These concentration values (higher than the maximum values that can be found among the population) were the following ones:

- **Adenovirus**: 30000 ng/ml, about 1000-fold its detection limit.
- **Rotavirus**: 50000 ng/ml, about 1600-fold its detection limit.
- **Norovirus GI**: 15000 ng/ml, about 1200-fold its detection limit.
- **Norovirus GII**: 5000 ng/ml, about 3300-fold its detection limit.

INTERFERING SUBSTANCES

The substances indicated in the below table at the concentration specified did not interfere with the results of the test when added to stool samples (positive and negative ones):

Racecadotril	5% (p/v)	Ibuprofen	20% (p/v)
Cimetidine	10% (p/v)	Acetylsalicylic acid	30% (p/v)
Loperamide	5% (p/v)	Edulcorant	5% (p/v)
Metronidazole	10% (p/v)	Palmitic acid	40% (p/v)
Omeprazole	3% (p/v)	Barium Sulfate	5% (p/v)
Ampicillin	15% (p/v)	Mucin	5% (p/v)

CROSS-REACTIVITY

The microorganisms listed, at the indicated concentration, did not interfere with the Rota-Adeno-Noro test results.

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.*

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PRESENTATION SYMBOLS USED FOR IVD COMPONENTS AND REAGENTS

	Manufactured by	IVD	For in vitro diagnostic use
	Do not re-use		Please read pack insert
Σ n	Contains sufficient for <n> tests		Dry storage
REF	Catalogue number		Store at
LOT	Lot number		Expiry date
CE	This product fulfills the requirements of Directive 98/79/EC on in vitro diagnostic medical devices	DIL	Dilution buffer