VITASSAY

Rotavirus+Adenovirus + Norovirus

Rapid test for the simultaneous qualitative detection of rotavirus, adenovirus and norovirus in human stool samples.

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Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Rotavirus + Adenovirus + Norovirus is a rapid, immunochromatographic assay for the simultaneous qualitative detection of rotavirus, adenovirus and norovirus in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of rotavirus, adenovirus and norovirus infection.

INTRODUCTION

Enteric viruses have been recognized as the most significant etiological agents of the disease, and four categories of viruses are being considered clinically relevant: group A rotavirus (family *Reoviridae*), norovirus (family *Caliciviridae*), adenovirus 40/41 (subgenus F), and astrovirus.

Rotavirus is the leading cause of severe dehydration in children <5 years of age.

Most rotavirus infections are community-acquired and transmitted by the feco-oral route and peak the winter season between November and February in temperate climates.

Adenovirus, initially recognized as a cause of respiratory disease, is associated also with gastrointestinal, ophthalmological, and neurological infections. Watery, non-bloody diarrhea typically precedes vomiting and children admitted to the hospital for adenovirus gastroenteritis are more likely to present diarrhea that usually lasts more than in rotavirus gastroenteritis (more than 5 days).

Norovirus represents the most common cause of gastroenteritis outbreaks and causes acute, self-limiting gastroenteritis in people from all age groups.

PRINCIPLE

Vitassay Rotavirus + Adenovirus + Norovirus is a qualitative immunochromatographic assay to make a presumptive diagnosis of rotavirus, adenovirus and/or norovirus infection.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against rotavirus.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against adenovirus.

Strip C: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against norovirus.

During the process, the sample reacts with the antibodies against rotavirus (strip A) and/or adenovirus (strip B) and/or norovirus (strip C), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is rotavirus positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A, if the sample is adenovirus positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip B and if the sample is astrovirus positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip C. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for all the strips).

The presence of these green lines (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Rotavirus + Adenovirus + Norovirus**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **10** tests/kit **Vitassay Rotavirus + Adenovirus + Norovirus** ▪ Instructions for use. ▪ **10** vials with diluent for sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer. ▪ Spatula.

SPECIMEN COLLECTION

Stool samples should be collected in clean and dry containers. Collect sufficient quantity of feces: 1-2 g or 1-2mL for liquid samples.

The samples can be stored in the refrigerator (2-8ºC/36-46.4ºC) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC/4ºF. The samples will be brought to room temperature before to testing.

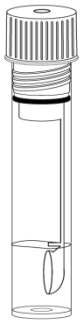
Homogenise stool sample as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1 Remove the cap of the vial with diluent for sample dilution (figure 1) and use the spoon to collect sufficient sample quantity. For solid stool, insert the spoon in 4 different areas of the stool sample (figure 2), remove any excess sample with a spatula (figure 3), and place the spoon cap back into the vial for sample dilution (figure 4). For liquid stool, take a spoonful of the sample (figure 3) and transfer it into the vial for sample dilution.

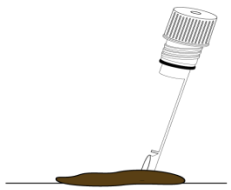
2.Close the vial for sample dilution tightly and shake it to dilute and mix the sample with the diluent (figure 4).

1



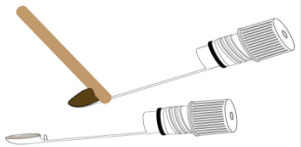
Vial for sample dilution

2



Insert the spoon in 4 different areas of the stool.

3



Remove the excess of sample with a spatula. Liquid samples: full spoon.

4

Put the sample into the vial, close the cap and shake.

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample vigorously to obtain a good sample dilution.

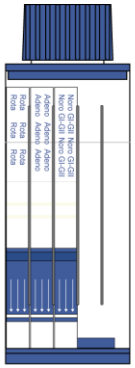
2. Remove the **Vitassay Rotavirus + Adenovirus + Norovirus** from its sealed bag just before using it (figure 5).

3. Take the vial containing the diluted sample (figure 6), place it inside the multiplex tube (figure 7). Screw the cap of the multiplex tube tightly (figure 8). The bottom of the collection tube will break and the diluent+sample solution reaches the sample zone of the strips (figure 9).

4. Leave the multiplex tube vertically on a flat surface and read the results at **10 minutes**. Do not read the test results later than 10 minutes.

If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.

5

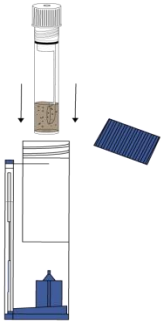


Vitassay Rotavirus+Adenvirus+ Norovirus

6

Vial with the diluted sample inside.

7



Introduce the vial with the diluted sample into the multiplex.

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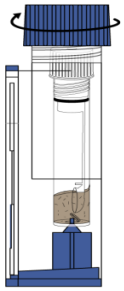
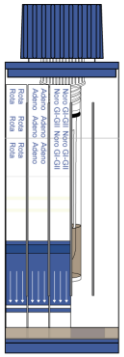
8



Close the cap and the bottom of the diluent vial will break.

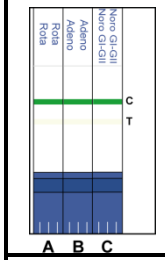
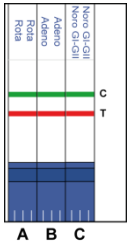
9

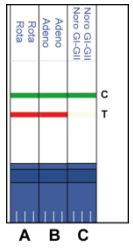
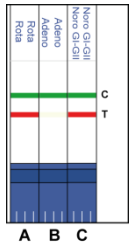
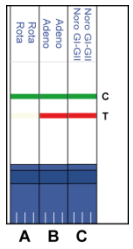
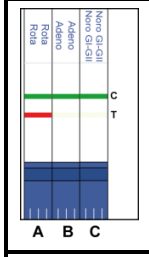
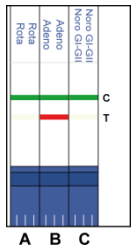


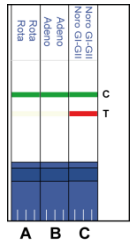
Reaction takes place. **Read results at 10 minutes.**

INTERPRETATION OF THE RESULTS

Strip A: rotavirus, Strip B: adenovirus, Strip C: norovirus

	**NEGATIVE** Only one green line in the control zone (C) in the three strips (A,B and C)	There is no rotavirus, adenovirus and norovirus presence.
	**POSITIVE** In addition to the green line (control line C), a red line appears in each strip, test line (T)	There is rotavirus, adenovirus and norovirus presence.

	**NEGATIVE** Strip C (norovirus)→ green line **POSITIVE** Strip A (rotavirus)→ green/red lines Strip B (adenovirus)→ green/red lines	There is rotavirus and adenovirus presence. Viral infection caused by rotavirus and adenovirus.
	**NEGATIVE** Strip B (adenovirus)→ green line **POSITIVE** Strip A (rotavirus)→ green/red lines Strip C (norovirus)→ green/red lines	There is rotavirus, nrovirus presence. Viral infection caused by rotavirus and norovirus.
	**NEGATIVE** Strip A (rotavirus)→ green line **POSITIVE** Strip B (adenovirus)→ green/red lines Strip C (norovirus)→ green/red lines	There is adenovirus and norovirus presence. Viral infection caused by adenovirus and norovirus.
	**NEGATIVE** Strip B (adenovirus)→ green line Strip C (norovirus)→green line **POSITIVE** Strip A (rotavirus)→ green/red lines	There is rotavirus presence. Viral infection caused by rotavirus.
	**NEGATIVE** Strip A (rotavirus)→ green line Strip C (norovirus)→ green line **POSITIVE** Strip B (adenovirus)→ green/red lines	There is adenovirus presence. Viral infection caused by adenovirus.

	**NEGATIVE** Strip A (rotavirus)→ green line Strip B (adenovirus)→ green line **POSITIVE** Strip C (norovirus)→ green/red lines	There is norovirus presence. Viral infection caused by norovirus.
Any other results	Invalid results either A, B or C, we recommend repeating the assay using the same sample with another test.	

Notes: The intensity of the red coloured test line in the result line region (T) will vary depending on the concentration of antigens in the specimen.

Positive results detailed in the above table should be followed up with additional confirmatory diagnostic procedures.

Single or dual simultaneous virus infections are more frequent than triple.

Invalid results: Total absence of any control coloured lines (green) indicates an invalid result, regardless of the appearance or not of the test lines (red**).** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the problem persists, discontinue using the kit and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in **Vitassay Rotavirus+Adenovirus+Norovirus**. Green lines appearing in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Rotavirus + Adenovirus + Norovirus** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- After one week of infection, the number of viruses in faeces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset symptoms.
- The use of other samples different from human fecal samples has not been established.
- The quality of **Vitassay Rotavirus + Adenovirus + Norovirus** depends on the quality of the sample; proper fecal specimens must be obtained.

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- Positive results determine the presence of rotavirus, adenovirus and/or norovirus in fecal samples. A positive result should be followed up with additional laboratory techniques to confirm the results. A confirmed infection should only be made by a physician after the evaluation of all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the detection limit value. If symptoms or situation still persist a rotavirus, adenovirus and/or norovirus determination should be carried out with another technique.

EXPECTED VALUES

Currently, rotavirus, norovirus, astrovirus and adenovirus 40/41 have been recognized as the most significant etiological agents of childhood viral gastroenteritis in industrialized countries.

In children, group A rotavirus is the major etiologic agent of viral gastroenteritis and is responsible for 29 to 45% of hospitalizations worldwide. Recent work has showed that noroviruses are the second most frequent etiologic agents of viral gastroenteritis in children.

In the European Union, it is estimated that 3.6 million episodes of rotavirus gastroenteritis occur annually. Rotavirus gastroenteritis is estimated to occur at a rate of 1 symptomatic infection in every 7 children each year, accounting for 231 deaths, more than 87000 hospitalizations, and almost 700000 outpatient visits. It has been estimated that rotavirus accounts for 39% diarrheal hospitalizations and from 25.3% to 63.5% of community-acquired acute gastroenteritis in children <5 years of age.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation was performed using **Vitassay Rotavirus + Adenovirus + Norovirus** and other commercial test (Ridascreen®Rotavirus ELISA Test, r-Biopharm).

Results were as follows:

		Ridascreen®Rotavirus ELISA Test		
		Positive	Negative	Total
Vitassay Rotavirus + Adenovirus + Norovirus Rotavirus	Positive	18	1	19
	Negative	0	43	43
	Total	18	44	62

Vitassay Rotavirus + Adenovirus + Norovirus (Rotavirus) **vs Ridascreen®Rotavirus ELISA Test**			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	98%	>94%	>99%

And evaluation was performed using **Vitassay Rotavirus + Adenovirus + Norovirus** and PCR.

Results were as follows:

		PCR		
		Positive	Negative	Total
Vitassay Rotavirus + Adenovirus + Norovirus Adenovirus	Positive	7	0	7
	Negative	0	52	52
	Total	7	52	59

Vitassay Rotavirus + Adenovirus + Norovirus (Adenovirus) **vs PCR**			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

And evaluation was performed using **Vitassay Rotavirus + Adenovirus + Norovirus** and other commercial test (Simple Norovirus, Operon).

Results were as follows:

		Simple Norovirus		
		Positive	Negative	Total
Vitassay Rotavirus + Adenovirus + Norovirus Norovirus GI	Positive	2	0	2
	Negative	0	48	48
	Total	2	48	50

Vitassay Rotavirus + Adenovirus + Norovirus (Norovirus GI) **vs Simple Norovirus**			
Sensitivity	**Specificity**	**VPP**	**VPN**
>99%	>99%	>99%	>99%

		Simple Norovirus		
		Positive	Negative	Total
Vitassay Rotavirus + Adenovirus + Norovirus Norovirus GII	Positive	10	0	10
	Negative	0	48	48
	Total	10	48	58

Vitassay Rotavirus + Adenovirus + Norovirus (Norovirus GII) **vs Simple Norovirus**			
Sensitivity	**Specificity**	**VPP**	**VPN**
>99%	>99%	>99%	>99%

The results showed that **Vitassay Rotavirus + Adenovirus + Norovirus** has a high sensitivity and specificity to detect rotavirus, adenovirus and norovirus.

Cross reactivity

No cross reactivity was detected against other gastrointestinal pathogens that are occasionally present in feces.

Adenovirus (Strip A and C)	*Giardia lamblia*	*Salmonella typhi*
Astrovirus	*Helicobacter pylori*	*Shigella boydii*
Campylobacter coli	*Hepatitis A*	*Shigella dysenteriae*
Campylobacter jejuni	*Listeria monocytogenes*	*Shigella flexneri*
Clostridium difficile	*Norovirus* (Strip A and B)	*Shigella sonnei*
Cryptosporidium parvum	*Rotavirus* (Strip B and C)	*Staphylococcus aereus*
Entamoeba hystolitica	*Salmonella enteritidis*	*Yersinia enterocolítica*
Enterovirus	*Salmonella paratyphi*	
Escherichia coli O157:H7	*Salmonella typhimurium*	

REFERENCES

1. ADISSA TRAN; DEBORAH TALMUD; BENOIT LEJEUNE; NICOLAS JOVENIN; FANNY RENOIS; CHRISTOPHER PAYAN; NICOLAS LEVEQUE; LAURENT ANDREOLETTI. "Prevalence of Rotavirus , Adenovirus, Norovirus and Astrovirus infections and coinfections among hospitalized children in Nothern France". Journal of Clinical Microbiology, May 2010, p. 1943-1946.

2. D. DONA; E. MOZZO; A. SCAMARCIA; G. PICELLI; M. VILLA; L. CANTARUTTI; C. GIAQUINTO. "Community-Acquired Rotavirus Gastroenteritis compared with adenovirus and norovirus gastroenteritis in Italian children: a pedianet study". Hindawi Publishing Corporation – International Journal of Pediatrics Volume 2016, article ID 5236243, 10 pages.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

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