VITASSAY

Rotavirus+Adenovirus+ Astrovirus

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

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

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

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

INTRODUCTION

Acute gastroenteritis is a major cause of morbidity and mortality in pediatric populations worldwide. Globally, an estimated 800000 infants and young children die from diarrhea each year. Mortality is uncommon in developed countries, but diarrhea is often associated with substantial medical and healthcare costs and thus has a high economic impact in society.

Virus are the major etiological agents of acute gastroenteritis in children <5 years of age. Group A rotavirus, norovirus, enteric adenovirus, human astrovirus, and sapovirus are established etiological agents of acute gastroenteritis.

PRINCIPLE

Vitassay Rotavirus+Adenovirus+Astrovirus is a qualitative immunochromatographic assay to make a presumptive diagnosis of rotavirus, adenovirus and/or astrovirus 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 astrovirus.
During the process, the sample reacts with the antibodies against rotavirus (strip A) y/o adenovirus (strip B) and/or astrovirus (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. 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 a green line (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. Single use device.
- 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+Astrovirus**. 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.

MATERIALS

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

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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.

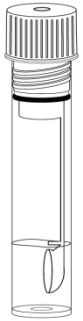
The samples can be stored in the refrigerator (2-8ºC/36-46.4ºC) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC/4ºF. The samples will be brought to room temperature before to testing.

Homogenise stool sample as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

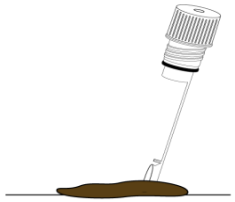
1. Remove the cap of the vial with diluent for sample dilution (figure 1) and use the spoon to collect sufficient sample quantity. For solid stool, insert the spoon in 4 different areas of the stool sample (figure 2), remove any excess sample with a spatula (figure 3), and place the spoon cap back into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



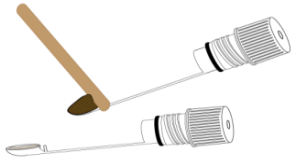
Vial for sample dilution.

2



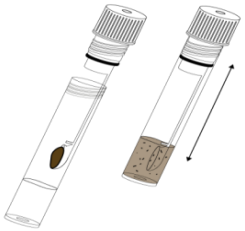
Insert the spoon in 4 different areas of the stool.

3



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

4



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

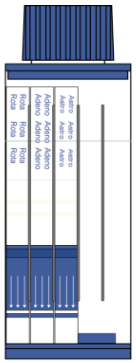
PROCEDURE

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+Astrovirus** from its sealed bag just before using it (figure 5).
3. Take the vial for sample dilution 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 vial for sample dilution 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.

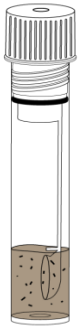
If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.

5



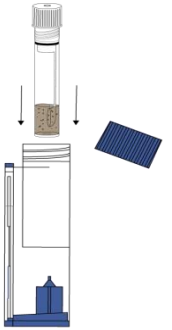
Vitassay Rotavirus+Adenovirus+ Astrovirus.

6



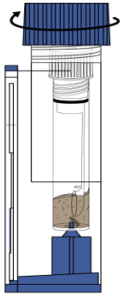
Vial with the diluted sample inside.

7



Introduce the vial with the diluted sample into the multiplex.

8



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

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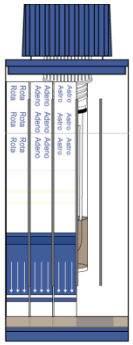
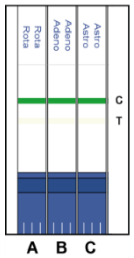
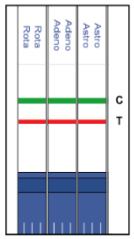
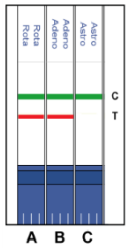
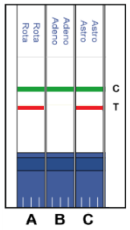
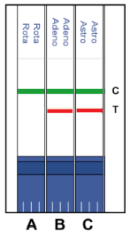
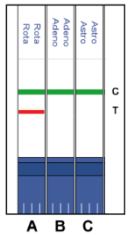
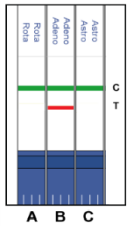
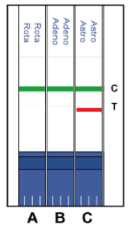
9



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

INTERPRETATION OF THE RESULTS

Strip A: rotavirus, Strip B: adenovirus and Strip C: astrovirus

	**NEGATIVE** Only one green line in the control zone (C) in the three strips (A,B and C)	There is no rotavirus, adenovirus and astrovirus 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 astrovirus presence.
	**NEGATIVE** Strip C (astrovirus)→ 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 (astrovirus)→ green/red lines	There is rotavirus, astrovirus presence. Viral infection caused by rotavirus and astrovirus.
	**NEGATIVE** Strip A (rotavirus)→ green line **POSITIVE** Strip B (adenovirus)→ green/red lines Strip C (astrovirus)→ green/red lines	There is adenovirus and astrovirus presence. Viral infection caused by adenovirus and astrovirus.
	**NEGATIVE** Strip B (adenovirus)→ green line Strip C (astrovirus)→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 (astrovirus)→ 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 (astrovirus)→ green/red lines	There is astrovirus presence. Viral infection caused by astrovirus.
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+Astrovirus**. Green lines appearing in the in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Rotavirus+Adenovirus+Astrovirus** 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 feces 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+Astrovirus** depends on the quality of the sample; proper fecal specimens must be obtained.
- Positive results determine the presence of rotavirus, adenovirus, and/or astrovirus 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 astrovirus determination should be carried out with another technique.

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EXPECTED VALUES

According to recent estimates, nearly 179 million gastrointestinal illnesses occur each year in the United States, of which 141.8 million (approximately 80%) are caused by unspecified and/or unknown agents.

Diarrhea is responsible for 11% of deaths among children under five years old, and acute gastroenteritis is ones of the leading causes of death worldwide.

The incidence of diarrhea is higher in developing countries than in industrialized countries.

Rotavirus is a major cause of severe diarrhea in both developed and developing countries. Previous report estimates that more than 100 million episodes of gastroenteritis requiring home care only. However, almost 25 million cases need clinical visit, 2 million cases need hospital admission, and 440000 death cases in children under 5 years of age.

The rate of enteric adenovirus 40 and 41 varies from 1-8% in developed countries to 2-31% in developing countries, but the prevalence is increased in immunocompromised patients.

The prevalence rate of human astrovirus infection ranged from 2% to 9% among children with diarrhea, although incidences over 60% have also been reported. The morbidity varies depending on the season, with higher infection during the winter in temperate climates and the rainy season in tropical regions.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity-Rotavirus

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

Results were as follows:

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

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

Clinical sensitivity and specificity-Adenovirus

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

Results were as follows:

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

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

Clinical sensitivity and specificity- Astrovirus

And evaluation was performed using **Vitassay Rotavirus+Adenovirus+Astrovirus** and an Elisa assay (Ridasscreen@Astrovirus Test, r-Biopharm).

Results were as follows:

		Ridascreen®Astrovirus Test		
		Positive	**Negative**	**Total**
Vitassay Rotavirus+Adenovirus+ Astrovirus Astrovirus	**Positive**	16	0	16
	Negative	1	11	12
	Total	17	11	28

Vitassay Rotavirus+Adenovirus+Astrovirus (Astrovirus) **vs Ridascreen®Astrovirus Test**			
Sensitivity	**Specificity**	**PPV**	**NPV**
>94%	>99%	>99%	>92%

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

Cross reactivity

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

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

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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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