VITASSAY

Rotavirus+Adenovirus

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

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

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

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

INTRODUCTION

Viral pathogens are the most common cause of gastroenteritis in developed countries. Human rotavirus and adenovirus infections are major causes of acute outbreaks and sporadic cases of gastroenteritis, occurring primarily among children less than 2 years of age. Patient hospitalization is often required, with enormous infection control implications.

PRINCIPLE

Vitassay Rotavirus+Adenovirus is a qualitative immunochromatographic assay for the detection of rotavirus and adenovirus in human stool samples**.**

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.
During the process, the sample reacts with the antibodies against rotavirus (strip A) and/or adenovirus (strip B), 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, and 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. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for both 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. Device for single use.
- 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**. 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
▪ **25** tests/kit **Vitassay Rotavirus+Adenovirus** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

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

Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it

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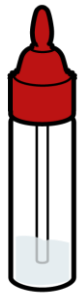
into the vial with diluent for the sample dilution. For liquid stool, take 125 µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.

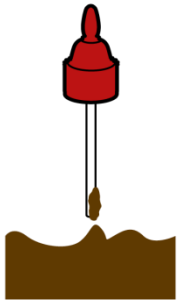
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).

1



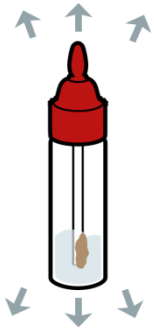
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

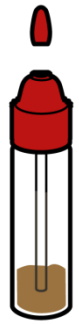
PROCEDURE

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** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter A – rotavirus (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter B – adenovirus (figure 6).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

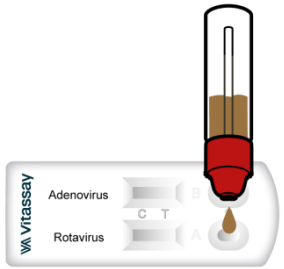
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



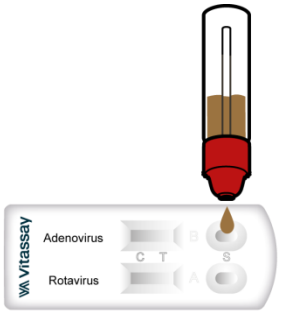
Cut the end of the cap.

5



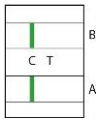
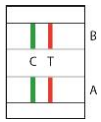
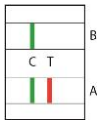
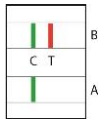
Dispense 4 drops in the circular window marked with the letter S to the strip A – Rotavirus.

6



Dispense 4 drops in the circular window marked with the letter S to the strip B – Adenovirus.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Rotavirus	Strip B Adenovirus	INTERPRETATION
	**Negative**	**Negative**	There is no rotavirus and/or adenovirus presence. There is not infection caused by rotavirus and/or adenovirus.
	GREEN	GREEN	
	**Positive**	**Positive**	There is rotavirus and adenovirus presence. Infection caused by rotavirus and adenovirus.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is rotavirus presence. Infection caused by rotavirus.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is adenovirus presence. Infection caused by adenovirus.
	GREEN	GREEN-RED	
ANY OTHER RESULTS			Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the

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	reagents are the main reasons of control line failure. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor.

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

QUALITY CONTROL

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

LIMITATIONS

- **Vitassay Rotavirus+Adenovirus** 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** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of rotavirus and/or adenovirus in fecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods or microscopy) 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 and/or adenovirus determination should be carried out with another technique (as for example microscopy).

EXPECTED VALUES

Acute gastroenteritis is a worldwide health problem. It has also been reported as an important factor for childhood morbidity and mortality worldwide. Almost 1.76 million children under age 5 die annually from gastroenteritis in both developing and developed countries.

Group A rotaviruses (HRV) are the major cause of pediatric acute gastroenteritis worldwide followed, to a lesser extent, by enteric adenoviruses types 40 and 41 and other viral agents.

Globally, an estimated 702,000 children die each year from rotavirus diarrhea, the vast majority of whom are in developing countries. Children under 5 years of age are particularly prone, and infection is predominant among those aged 6-24 months.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

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

Results were as follows:

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

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

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

Results were as follows:

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

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

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

Cross reactivity

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

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

REFERENCES

1. CATRIONA LOGAN, JOHN J. O'LEARY and NIAMH O'SUILLIVAN. "Real-Time Reverse Transcription-PCR for Detection of Rotavirus and Adenovirus as Causative Agents of Acute Viral Gastroenteritis in Children". Journal of Clinical Microbiology, Vol. 44, No. 9, Sept. 2006, p. 3189-3195.

2. MARYAM REZAEI, AMIR SOHRABI, ROSITA EDALAT, SEYED DAVAR SIADAT, HOSNA GOMARI, MARZIYEH REZAEI, SHAHAB MODARRES GILANI. "Molecular Epidemiology of Acute Gastroenteritis Caused by Subgenus F (40, 41) Enteric Adenoviruses in Inpatient Children". LABMEDICINE, Vol. 43, No. 1, Jan. 2012, p. 10-15.

3. ANTONIO CARRATURO, VALENTINA CATALANI, LUCIANO TEGA. "Microbiological and epidemiological aspects of Rotavirus and enteric Adenovirus infections in hospitalized children in Italy" NEW MICROBIOLOGICA, 31, 329-336, 2008.

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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