

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.

- Read the instructions for use carefully before using the test.
- Do not use the kit if the label sealing the outer carton is torn or if the bags are open or damaged on arrival.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- Specimens should be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations. A new test should be used for each sample to avoid contamination errors.
- Material exposed to the samples should also be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations.
- Do not reuse. This is a single-use device.
- Tests and used material should be disposed of in an appropriate 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 or components from other batches.
- Follow Good Laboratory Practices. These practices should include, but are not limited to, personal protective equipment (PPE), such as lab coat, surgical or appropriate mask or face shield, disposable gloves and eye protection. Take the necessary precautions during sample collection, transport, storage, handling and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink or smoke in the workplace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.
- The visual interpretation of the results is done by coloured lines, the interpretation of the results should be done by a professional user without problems of visualisation and colour interpretation.
- A certificate of analysis can be provided on request (not included).

STORAGE AND STABILITY

The storage temperature of the kits should be 2-30°C.

Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

VITASSAY

Rotavirus+Adenovirus

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

IUE-7455010 Ed02 October 2023



MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none"> 25 tests/kit Vitassay Rotavirus+Adenovirus Instructions for use. 25 vials with diluent for the sample dilution. 	<ul style="list-style-type: none"> PPE, such as disposable gloves Specimen collection container Timer Micropipette (in case of liquid stool)

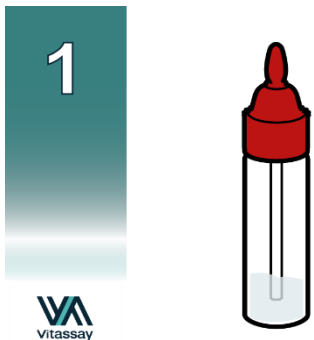
SPECIMEN COLLECTION

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

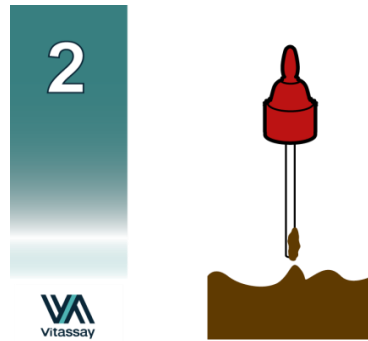
Samples can be stored in the refrigerator (2-8°C) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C. Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

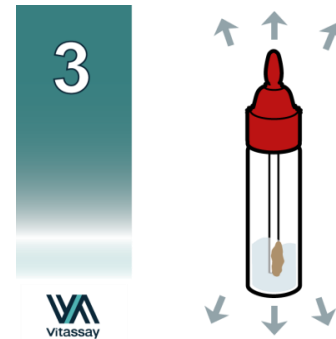
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for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



Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



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

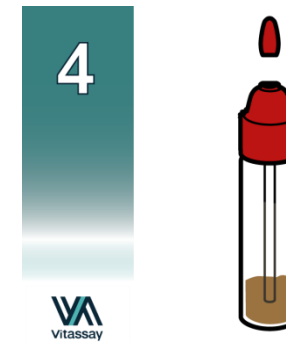
PROCEDURE

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

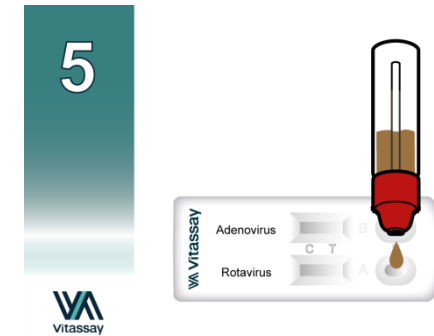
1. Shake the vial with the sample vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Rotavirus+Adenovirus** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked 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,

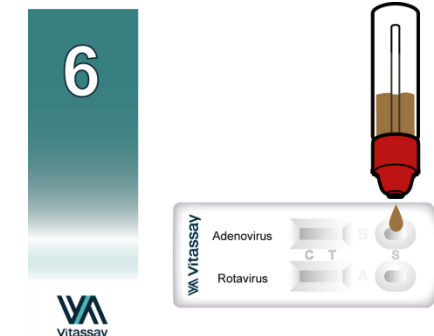
dispense a drop of diluent until seeing the liquid running through the reaction zone.



Cut the end of the cap

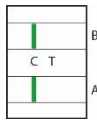

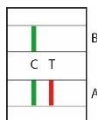
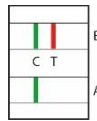


Dispense 4 drops in the circular window marked with the letter S to the strip A – Rotavirus.



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 GREEN	Negative GREEN	There is no rotavirus and/nor adenovirus presence. Apparently, there is no infection caused by rotavirus and/or adenovirus.
	Positive GREEN-RED	Positive GREEN-RED	There is rotavirus and adenovirus presence. Possible infection caused by rotavirus and adenovirus.
	Positive GREEN-RED	Negative GREEN	There is rotavirus presence. Possible infection caused by rotavirus.
	Negative GREEN	Positive GREEN-RED	There is adenovirus presence. Possible infection caused by adenovirus.
ANY OTHER RESULTS			Invalid result, we recommend repeating the assay using the sample with another test. Note: Wrong procedural techniques, insufficient sample volume or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation 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

- 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.
- The use of other samples different from human samples has not been established.
- After one week of infection the presence of virus shed in stool decreases considerably so a lower concentration in the stool sample is probable. The stool sample should be taken within the first week of symptom onset.
- 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 adenovirus and/or rotavirus in fecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods or PCR) to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated 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 antigens in the faecal sample 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 (for example PCR).
- **Bloody stool samples** and/or **mucous stool samples** can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the result.

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) for the strip A.

Results were as follows:

		Ridascreen® <i>Rotavirus</i> ELISA Test		
		Positive	Negative	Total
Vitassay Rotavirus + Adenovirus	Positive	18	1	19
	Negative	0	43	43
	Total	18	44	62

Table 1. Results of **Vitassay Rotavirus+Adenovirus (Rotavirus)** compared to a commercial kit.

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

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Adenovirus (Rotavirus)** compared to a commercial kit.

A second evaluation was performed using **Vitassay Rotavirus+Adenovirus** and PCR for the strip B.

Results were as follows:

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

Table 3. Results of **Vitassay Rotavirus+Adenovirus (Adenovirus)** confirmed by qPCR technique.

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

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Rotavirus+Adenovirus (Adenovirus)** confirmed by qPCR technique.

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 faeces:

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

REFERENCES

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- 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.
- 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		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
	CE Marking		

Changes control		
Nº Version	changes	Date
IUE-7355010 Ed02 October 2023	The format has been updated. A transcription error in the interpretation section has been corrected. The limitations section has been updated. Grammatical and editorial changes have been made to the Precautions, Limitations, Sample Collection, Storage and Stability sections. Required but not included material updated with minor changes. The wording has been changed under Interpretation of results.	03/10/2023

