VITASSAY

Adenovirus

Rapid test for the qualitative detection of Adenovirus in human stool samples.

IUE-7355009 Ed01 October 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Adenovirus is a rapid one step immunochromatographic assay for the qualitative detection of Adenovirus in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of Adenovirus infection.

INTRODUCTION

Human Adenovirus is a double stranded DNA virus, non-enveloped, that belongs to the *Mastadenovirus* genus within *Adenoviridae* family. The infections caused by human Adenovirus may be asymptomatic, or may induce common diseases such as respiratory distress, gastroenteritis and hemorrhagic cystitis.

Rotavirus, Adenovirus and norovirus are often related with both diarrhoea and subclinical infections. These viruses are shed in the faeces of infected (symptomatic and asymptomatic) individuals and acquired through the faecal-oral route by the consumption of contaminated water, food, direct contact and aerosols. The enteric viruses are a special concern in public health due to their wide distribution, rapid transmission, high prevalence, and resistance under environmental conditions.

Risk of outbreaks is higher in Adenovirus infections because faecal viral breakthrough continue for a long time after diarrhoea is over.

PRINCIPLE

Vitassay Adenovirus is a qualitative immunochromatographic assay for detection of Adenovirus in human stool samples.

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 Adenovirus, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this 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.
- 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 must 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 Adenovirus**. Do not use any other commercial kit component or components from other batched.
- 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).

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STORAGE AND STABILITY

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

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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ 25 tests/kit **Vitassay Adenovirus** ▪ Instructions for use. ▪ 25 vials with diluent for sample dilution.	▪ PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer ▪ Micropipette (in case of liquid stool)

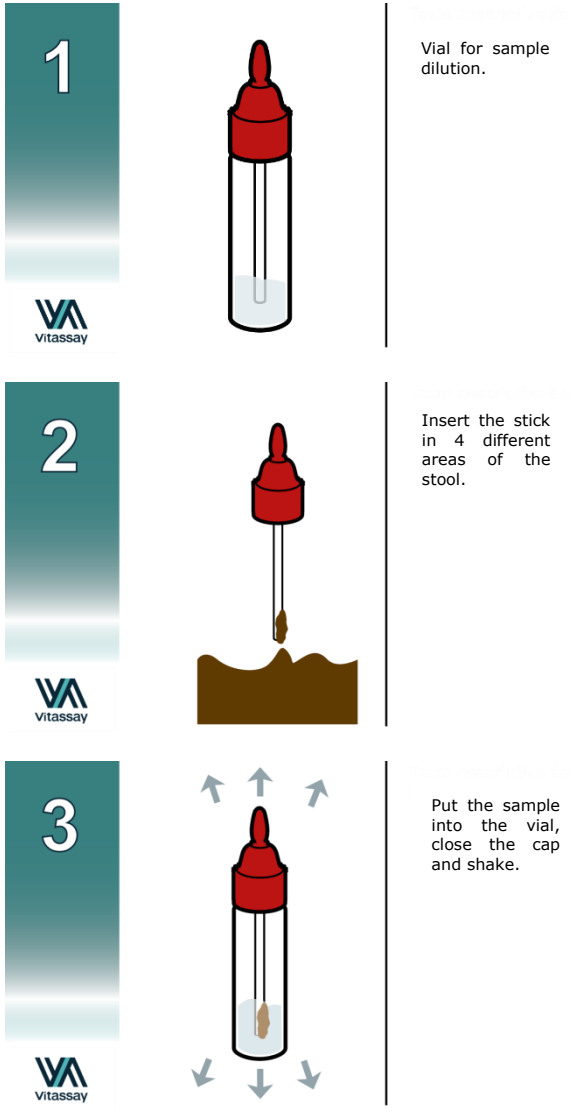
SPECIMEN COLLECTION

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

SPECIMEN PREPARATION

1. Remove the cap of the vial with diluent for sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick once 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 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake the vial vigorously in order to assure good sample dispersion (figure 3).

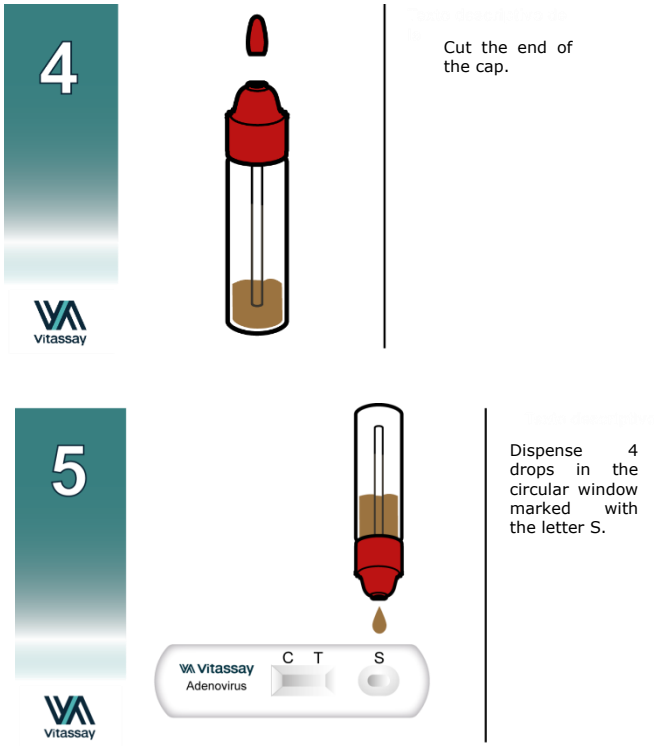


PROCEDURE

Allow the test, stool sample, controls and diluents to reach room temperature (15-30ºC) 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 Adenovirus** from its sealed bag just before using it.
3. Take the vial for sample dilution containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter S (figure 5).
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 circular window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



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INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C)**	There is no presence of adenovirus. No apparent infection caused by Adenovirus.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears, **test line(T)**	There is presence of adenovirus. Possible viral 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 control is included in **Vitassay Adenovirus**. Green line appearing in the results window is an internal control, which confirms 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 Adenovirus** depends on the quality of the sample. Proper faecal specimens must be obtained.
- Positive results determine the presence of adenovirus in faecal 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, an 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

Infectious diarrhoea is one of the most common diseases affecting children <5 years old, leading to significant morbidity and mortality worldwide, especially in developing countries. Diarrhoea causes >1.8 million deaths each year. Although many pathogens can cause diarrhoea, >75% of cases are caused by viruses. Rotavirus are the leading cause of severe diarrhoea among children <5 years of age. Sapoviruses, astroviruses, and Adenoviruses have also been reported to cause diarrhoea in children.

Adenovirus usually accounts for 3.2 to 12.5% of acute diarrhoea cases, and the detection ratio is higher in developing countries than in developed countries.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with faecal samples was performed using **Vitassay Adenovirus** and confirmed by PCR.

Results were as follows:

		PCR		
		Positive	**Negative**	**Total**
Vitassay Adenovirus	**Positive**	7	0	7
	Negative	0	52	52
	Total	7	52	59

Table 1. Results of **Vitassay Adenovirus** compared to a commercial kit and confirmed with PCR.

.Vitassay Adenovirus vs PCR			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Adenovirus** confirmed with PCR.

Subsequently, another **Vitassay Adenovirus** evaluation was performed, where discrepant results were confirmed with qPCR technique.

Results are shown below:

		Simple Rota-Adeno (qPCR as reference technology)		
		Positive	**Negative**	**Total**
Vitassay Adenovirus	**Positive**	94	1	95
	Negative	4	148	152
	Total	98	149	247

Table 3. Results of **Vitassay Adenovirus** compared to a commercial kit and confirmed with PCR.

Vitassay Adenovirus vs Simple Rota-Adeno			
Sensibilidad	**Especificidad**	**VPP**	**VPN**
95.9% (89.9-98.9%)	99.3% (96.3-100%)	98.9% (94.3-100%)	97.4% (93.4-99.3%)

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Adenovirus** compared to a commercial kit and confirmed with PCR.

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

Cross reactivity

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

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

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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
CE	CE Marking		

Changes control		
Nº Version	**changes**	**Date**
IUE-7355009 Ed00 May 2016	Original version	05/2016
IUE-7355009 Ed01 October 2023	Addition of a new evaluation. 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

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