VITASSAY

HAV

Rapid test for the qualitative detection of Hepatitis A virus in human stool samples.

IUE-7355019 Ed00 July 2016

CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay HAV is a rapid one step immunochromatographic assay for the qualitative detection of Hepatitis A virus in human stool samples.

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

INTRODUCTION

Hepatitis A virus (HAV) is a small, non-enveloped hepatotropic virus classified into *Hepatovirus* genus within the *Picornaviridae* family.

HAV infection is the leading worldwide cause of acute viral hepatitis.

HAV is transmitted mainly by the fecal-oral route, either by person-to-person contact or by ingestion of contaminated water and food, particularly shellfish, soft fruits and raw vegetables.

HAV infection may be asymptomatic or may range in severity from a mild illness lasting 1-2 weeks to a severity disabling disease lasting several months to fulminant hepatitis. The severity of symptoms increases with age. Fulminant hepatitis occurs rarely (<1% overall), but rates are higher with increasing age and in the presence of underlying chronic liver disease, including chronic hepatitis B or C infection.

PRINCIPLE

Vitassay HAV is a qualitative immunochromatographic assay for the detection of Hepatitis A virus in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against HAV.
During the process, the sample reacts with the antibodies against HAV, 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.
- 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 HAV**. 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 HAV.** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

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

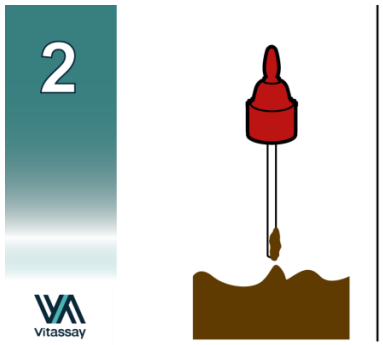
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing.

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

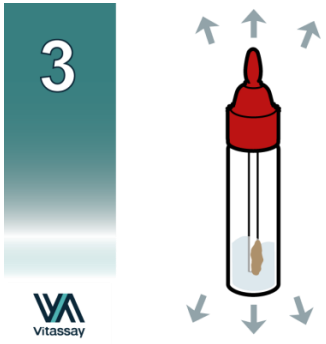
SPECIMEN PREPARATION

1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. 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 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 vigorously the tube in order to assure good sample dispersion (figure 3).

Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



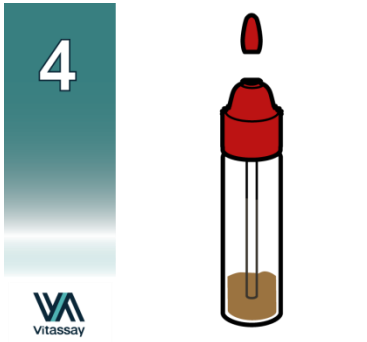
Put the sample into the vial, close the cap and shake.

PROCEDURE

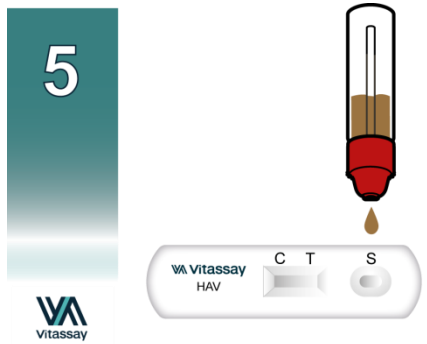
Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay HAV** from its sealed bag just before using it.
3. Take the stool collection tube 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 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.

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C).**	There is no HAV presence. No infection caused by HAV.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears (**test line T**).	There is HAV presence. Viral infection caused by HAV.
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for 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 control is included in **Vitassay HAV**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

LIMITATIONS

- **Vitassay HAV** 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.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay HAV** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of HAV 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 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 is lower than the detection limit value. If symptoms or situation still persist, an Hepatitis A virus determination should be carried out with another technique.

EXPECTED VALUES

There are an annual estimated of 1.5 million of cases of Hepatitis A worldwide.

HAV's geographical distribution is dependent on socioeconomic development and sanitation levels.

In areas with high and very high endemicity (Africa, Middle East, India, Central and South America), where infections are mostly asymptomatic and epidemics are rare, 50% seroprevalence is reached between the ages of 5 and 14.

In areas with moderate endemicity (Eastern Europe and south eastern Asia), 50% seroprevalence is reached between the ages of 14 and 34 and epidemics can occur within the general population.

In areas with low endemicity (North America, Western Europe and Australia), most of the population is still susceptible to HAV, particularly people over 50 years old, and the risk of fulminant hepatitis is higher.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with fecal samples was performed using **Vitassay HAV** and another commercial HAV immunoassay (HAV-Antigen EIA, Mediagnost®).

Results were as follows:

		HAV-Antigen EIA		
		Positive	Negative	Total
Vitassay HAV	Positive	2	0	2
	Negative	0	24	24
	Total	2	24	26

Vitassay HAV vs HAV-Antigen EIA			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

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

Cross reactivity

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

Adenovirus	*Enterovirus*	*Rotavirus*
Astrovirus	*Norovirus*	

REFERENCES

1. CORALIE COUDRAY-MEUNIER; AUDREY FRAISSE; CAMELIA MOKHTARI; SANDRA MARTIN-LATIL; ANNE-MARIE ROQUE-AFONSO; SYLVIE PERELLE. "Hepatitis A virus subgenotyping on RT-qPCR assays". BMC Microbiology 2014, 14:296.

2. GIUSEPPINA LA ROSA; SIMONETTA DELLA LIBERA; MARCELLO LACONELLI; ANNARITA CICCAGLIONE; ROBERTO BRUNI; STEFANIA TAFFON; MICHELE EQUESTRE; VALERIA ALFONSI; CATERINA RIZZO; MARIA ELENA TOSTI; MARIA CHIRONNA; LUISA ROMANO; ALESSANDRO REMO ZANETTI; MICHELE MUSCILLO. "Surveillance of hepatitis A virus in urban sewages and comparison with cases notified in the course of an outbreak, Italy 2013". BMC Infectious Diseases 2014, 14:419.

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

Vitassay

Vitassay

Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com