Hepatitis A Ag

Quick Test

Code 354 Hepatitis A Ag Kit
20 test devices
20 stool collection tubes

IVD
Store at 2 - 30ºC.

CYPRESS DIAGNOSTICS

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Intended Use :
Cypress Diagnostics' Hepatitis A Ag test is a rapid, one-step, visual immunochromatographic test for the qualitative detection of Hepatitis A virus antigen in faecal samples. This test is intended to aid in the diagnosis of acute hepatitis A infection.

Introduction :
Hepatitis A is a liver infection caused by the Hepatitis A virus (HAV). This virus is spread through faecal contamination of food or drinking water. The symptoms of Hepatitis A range from mild to severe, and can include fever, malaise, loss of appetite, diarrhoea, nausea abdominal discomfort, dark-coloured urine and jaundice. The severity of acute HAV infections is depending on the age of the patient, with younger patients tending to have milder symptoms than the elderly.
The immune response to an acute HAV infection includes the production of IgM antibodies. However, the blood IgM antibody level is still very low at the appearance of the symptoms and peaks only one to two weeks later.
The virus itself is secreted in the faeces from 1-2 weeks before the onset of illness to 1 to 2 weeks thereafter. Therefore, tests based on the detection of HAV antigen in faeces are very suitable to screen patients at the time the clinical symptoms appear. Moreover, such test can also be used previously to the onset of the illness in case HAV infection is suspected.

Principle :
The Hepatitis A Ag test is a qualitative lateral flow immunoassay. The membrane is pre-coated with mouse monoclonal antibodies against hepatitis A viral antigens on the test line region.
During testing, the sample is allowed to react with the coloured conjugate, containing anti-Hepatitis A virus antibodies, which was pre-dried on the test strip. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will react with the conjugate mixture and generate a coloured line. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a GREEN coloured band always appears. The presence of this GREEN band serves as verification that sufficient volume is added, that proper flow is obtained and as an internal control for the reagents.

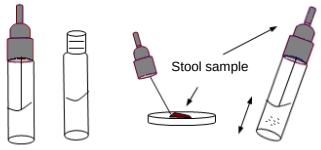
Kit components:
Each kit contains following items:
- 20 Cypress Diagnostics' Hepatitis A Ag test devices, individually wrapped in foil pouch with desiccant
- 20 stool collection tubes with sample diluent
- One instruction for use

Materials required but not provided:
- Sample collection container.
- Timer.

Storage and stability :
Store as packaged in the sealed pouch at 2-30ºC. The test is stable through the expiration date printed on the packaging. The test must remain in the sealed pouch until use. Do not freeze.

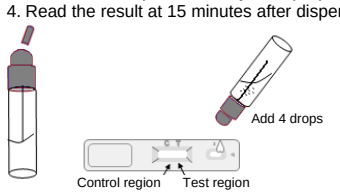
Precautions :
- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- All the samples should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.

Sample collection and preparation :
Stool samples should be collected in clean and dry containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-8ºC) for 1-2 days prior to testing. For longer storage , maximum 1 year, the samples must be kept frozen at –20ºC. In this case, the sample should be totally thawed, and brought to room temperature before testing.
Homogenise stool samples as thoroughly as possible prior to preparation.
Use a separate stool collection tube for each sample or control.
1. Unscrew the cap of the stool collection tube and introduce the stick in 3 different parts of the sample to pick up the sample (approx. 150 mg). If the stool sample is liquid, take 150 µl using a pipette and add the sample to the collection tube.
2. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion.



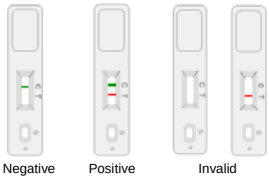
(1) (2)

Test Procedure :
1. Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC) prior to testing. Do not open the pouch until ready to perform the assay.
2. Remove the Hepatitis A Ag test from its sealed pouch just before using.
3. Shake the stool collection tube to assure good sample dispersion. Break off the cap of the tube. Dispense exactly 4 drops (100 µl) into the circular sample well (S).
4. Read the result at 15 minutes after dispensing the sample.



(3) (4)

Interpretation of the results :



NEGATIVE: Only one GREEN line appears in the result window at the control region marked with the letter C. A negative result indicates no HAV infection.

POSITIVE: Two lines appears in the result window: a RED test line in the test region marked with the letter T and a GREEN control line in control region marked with the letter C. This result indicates a recent and contagious infection with HAV.

INVALID: A total absence of the green control line regardless the appearance or not of the red test line. Insufficient sample volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit.

Note: The intensity of the red test line in the test region (T) will vary depending on the concentration of viral antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

Quality control :
Internal procedural controls are included in the test. A green line appearing in the control region (C) confirms sufficient specimen volume and correct procedural technique. A clear background in the membrane is considered as an internal negative procedural control. If the test is working properly, the background in the result window should be clear and not interfere with the ability to read the test.

Limitations :
1. The test must be carried out within 2 hours of opening the sealed bag.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. After appearance of the clinical symptoms, the number of viruses in faeces is decreasing, making the sample less reactive. Faecal samples should be collected previously or around to the onset of symptoms.
4. This test provides a presumptive diagnosis of HAV infection. A confirmed infection diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated. It must be based in the correlation of the results with further clinical observations.

Performance Characteristics:
Sensitivity and specificity
26 stool samples were tested using the Cypress Diagnostics' Hepatitis A Ag test and all the results were confirmed by HAV-Antigen EIA assay (Mediagnost®). The results showed >99% of sensitivity and >99% of specificity.
The antibodies used to elaborate this test recognise HAV epitopes found in stool patients.
This preliminary values has to be taken with precaution until more evaluation data will be available.

Cross-reactivity
An evaluation was performed to determine the cross reactivity of Cypress Diagnostics' Hepatitis A Ag test. No cross reactivity was found with common intestinal pathogens, other organisms and substances occasionally present in faeces: *Rotavirus, Adenovirus, Astrovirus, Enterovirus*, *Norovirus.*

References
1. Minuk, G.Y. et al. *HPB*, 2005; 7: 56-64.
2. Bruguera, M. et al. *Enferm Infect Microbiol Clin. 2006;24:649-56.*

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