HAV Ag CARD

Ref. C-73

For in *Vitro* diagnostic use only

Immunochromatographic rapid test for the qualitative detection of Hepatitis A in faeces

I. INTRODUCTION AND INTENDED USE

The HAV Ag CARD is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis A antigens in faecal samples to aid in the diagnosis of HAV infection.

The Hepatitis A virus is a single-stranded RNA virus that belongs to the Picornaviridae family of viruses. The majority of acute HAV infections are subclinical. When symptoms do appear, they tend to be mild and non-specific in nature. Most commonly they include fever, general malaise, fatigue, abdominal discomfort and change in bowel habits. When severe, dark urine, pale stool and jaundice may appear. The severity of acute HAV infections is proportional to the age of the patient, with younger patients tending to have milder disease than the elderly.

HAV is spread through faecal contamination of food or drinking water. Although the virus is present in blood, the limited amount of circulating virus and short duration of viremia render parenteral transmission of this virus extremely uncommon. Faeces of infected individuals tend to contain the virus for a 2-week period prior to the onset of illness and for at least 2 weeks and perhaps as long as 3 months thereafter.

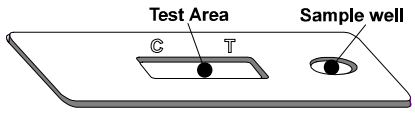
Acute hepatitis A is one of the well known vaccine preventable diseases and active Hepatitis A virus (HAV) vaccination is recommended in high risk populations.

HAV Ag CARD provides a rapid detection of Hepatitis A directly from the faecal samples.

II. PRINCIPLE OF THE TEST

The HAV Ag CARD is a qualitative lateral flow immunoassay for the detection of Hepatitis A virus antigen in faecal samples. 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 particle conjugate coated with 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 mixture conjugate 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.



III. REAGENTS AND MATERIALS

Each kit contains:

1. HAV Ag CARD device (20 items)
2. Extraction tubes with buffer (1mL x 20 tubes)
3. Instruction for use (1)

Required materials (not supplied)

Specimen collection container, disposable gloves, plastic pipette and timer.

IV. SPECIAL PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens 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.
- The test must be carried out within 2 hours of opening the sealed bag.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMENS COLLECTION FOR STOOL SAMPLES

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. PROCEDURE FOR STOOL SAMPLES

To process the collected stool samples

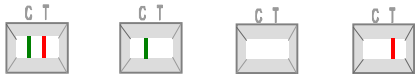
Use a separate tube with extraction buffer for each sample. Introduce the stick two times into the faecal specimen to pick up a little sample (150mg) and put into the testing tube with buffer. Shake the testing tube in order to assure good sample dispersion. For liquid stool samples, aspirate the faecal specimen with a dropper and add 150µL into the testing tube with buffer.

Test Procedure

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the card from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample.
3. Extract some liquid from the topside with a dropper. Dispense 4 drops or 100uL into the specimen well. Start the timer.
4. Read the result at **15 minutes** after dispensing the sample.

X. INTERPRETING THE RESULTS



Positive Negative Invalid

POSITIVE: Two lines appears across the central window in the result line region, a red test line marked with the letter T and in the control line region, a green control line marked with the letter C.

NEGATIVE: Only one green band appears across the control line region marked with the letter C.

INVALID: A total absence of the green control coloured band regardless the appearance or not of the red test line. Note: Insufficient specimen 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 and contact you local distributor.

The intensity of the red coloured band in the result line 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.

XI. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

XII. PERFORMANCE

A. Expected Values

The severity of acute HAV infections is proportional to the age of the patient, with younger patients tending to have milder disease than the elderly. Indeed, overall mortality rates are only 0.1% in the general population as opposed to 1–2% in the elderly.

B. Sensitivity and Specificity

It was studied 26 stool samples using HAV Ag CARD test and all of them 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.

C. Cross-Reactivity and interferences

It was performed an evaluation to determine the cross reactivity of HAV Ag CARD. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *Rotavirus, Adenovirus, Astrovirus, Enterovirus, Norovirus.*

XIII. LIMITS OF THE KIT

- The test must be carried out within 2 hours of opening the sealed bag.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some faecal samples can decrease the intensity of the control line.
- After one month of infection, the number of viruses in faeces is decreasing, making the sample less reactive. Faecal samples should be collected previously to the onset of symptoms to stop the spread of viruses.
- 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.

XIV. REFERENCES (See Italian version)

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non sterile
	Consult Instructions for Use		Use By (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

Fabbricante
INTERMEDICAL s.r.l.
Via A. Genovesi,13
80010 Villaricca(Na)-ITALY

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