OnSite CE

REF R0092C IVD In vitro Diagnostic

INTENDED USE

The HAV IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of antibodies (IgG and IgM) to hepatitis A virus (HAV) in human serum, plasma or whole blood. It is intended to be used as a screening test by professionals and provide a preliminary result to aid in the diagnosis of active and/or past HAV infection.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

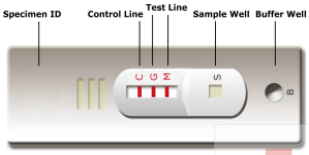
SUMMARY AND EXPLANATION OF THE TEST

HAV, a positive-sense RNA virus, is a unique member of the *Picornaviridae* family[1]. HAV is highly contagious and is primarily transmitted by the fecal-oral route, either through person to person contact or consumption of contaminated food or water. Although hepatitis A is not ordinarily a sexually transmitted disease, the infection rate can increase following oral-anal contact[2,3].

The presence of anti-HAV IgM in blood samples suggests an acute or recent HAV infection[4-6]. In most infected individuals, anti-HAV IgM rapidly increases in titer over a period of 4-6 weeks post-infection, and then declines to non-detectable levels within 3 to 6 months[7]. Anti-HAV IgG can be detected at the onset of symptoms, and levels remain elevated throughout the life of an individual. Protective immunity from an infection with HAV is indicated by an anti-HAV IgG level ≥20-33 mIU/mL[8], however these levels do not necessarily ensure protection from a future HAV infection. A patient without protective levels of anti-HAV IgG (< 20-33 mIU/mL) is considered at risk of acquiring an HAV infection.

The HAV IgG/IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of anti-HAV IgG (LoD 70 mIU/mL) and IgM in serum, plasma or whole blood. Results can be obtained within 15 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The HAV IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette device consists of: 1) a burgundy colored conjugate pad containing HAV antigens conjugated with colloidal gold (HAV conjugates) and a control antibody conjugated with colloidal gold; 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of anti-HAV IgG. The M line is pre-coated with mouse anti-human IgM for detection of anti-HAV IgM. The C line is pre-coated with a control antibody.

When an adequate volume of test specimen and sample diluent are dispensed into the sample and buffer wells, respectively, the specimen migrates by capillary action across the test strip. If anti-HAV IgG is present in the specimen, it will bind to the HAV conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a burgundy colored G line, indicating an HAV IgG positive test result. If anti-HAV IgM is present in the specimen it will bind to the HAV conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgM forming a burgundy colored M line, indicating an HAV IgM positive test result.

Absence of any test lines (G or M) suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies, regardless of color development on the test lines (G and M). If no control line (C line) develops, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (REF SB-R0092, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive control
2. Negative control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Lancing device for whole blood testing

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch until ready to conduct the assay.
3. Do not use expired devices or components.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use components from any other test kit as a substitute for components in this kit.
6. Do not use hemolyzed blood for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other bloodborne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read 15-20 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside 15-20 minutes should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused and unopened devices at 2-30°C. If stored at 2-8°C, ensure that the device is brought to room temperature before opening. The device is stable through the expiration date printed on the sealed pouch. Do not freeze or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.

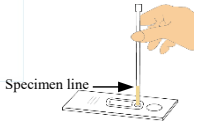
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the device on a clean, flat surface.

Step 3: Be sure to label the device with the specimen's ID number.

Step 4: Fill the capillary tube with specimen not exceeding the specimen line as shown in the images below. The volume of the specimen is approximately 5 µL. ***For maximum precision, transfer the specimen using a pipette capable of delivering a volume of 5 µL.***

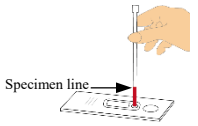
Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well (**S well**), making sure that there are no air bubbles.

Immediately add 2 drops (approximately 60-80 µL) of sample diluent into the buffer well (**B well**) with the bottle positioned vertically.



5 µL serum/plasma to S well **2 drops of sample diluent to B well**

Result
15 minutes



5 µL whole blood to S well **2 drops of sample diluent to B well**

Step 5: Set up timer.

Step 6: Read results at 15 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, any results interpreted outside 15-20 minutes should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.***

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. **External Control**: Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing the testing of the specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature during storage of the kits falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C line develops, the test indicates that anti-HAV antibodies are not detected in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT:**

2.1 In addition to the presence of the C line, if only the G line develops, the test result indicates

the presence of anti-HAV IgG. The result is HAV IgG positive or reactive, suggesting past infection.

2.2 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-HAV IgM. The result is HAV IgM positive or reactive, suggesting active infection.

2.3 In addition to the presence of C line, if both the G and M lines develop, the test indicates the presence of anti-HAV IgG and anti-HAV IgM. The result is HAV IgG and HAV IgM positive or reactive, suggesting active infection.

Specimens with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made. Rheumatoid factor levels ≥1,000 IU/mL may lead to unexpected positive results. *See Limitations of Test section, Number 6.*

3. INVALID RESULT: If no C line develops, the result is invalid regardless of any color development on the test lines (G and M) as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity of IgG Detection

The 2nd WHO International Standard for HAV (NIBSC 97/646) was reconstituted in water to 98 IU/mL and diluted with negative serum to concentrations of 40, 50, 60, 70, 80, 90, and 100 mIU/mL. Twenty replicates were tested on the HAV IgG/IgM Rapid Test. Defined as the 95% detection level, the limit of detection, or sensitivity, of the HAV IgG/IgM Rapid Test for the G test line is 70 mIU/mL.

HAV IgG (mIU/mL)	40	50	60	70	80	90	100
Number Positive	0	2	7	19	20	20	20
Number Negative	20	18	13	1	0	0	0

N=20, analytical sensitivity at 70 mIU/mL = 19/20 x 100% = 95%

2. Accuracy of IgG Detection

A total of 200 clinical specimens were collected and tested with the HAV IgG/IgM Rapid Test and with a reference commercial test kit. Comparison for all subjects is shown in the following table:

	HAV IgG/IgM Rapid Test		
Reference Test	Positive	Negative	Total
Positive	125	0	125
Negative	4	71	75
Total	129	71	200

Relative Sensitivity: 100.0% (95% CI: 97.1 - 100.0%)
Relative Specificity: 98.0% (95% CI: 95.0 - 99.5%)
Overall Agreement: 98.3% (95% CI: 96.7 - 99.3%)

3. Accuracy of IgM Detection

A total of 306 specimens were collected and tested with the HAV IgG/IgM Rapid Test and by a reference commercial anti-HAV IgM ELISA. Comparison for all subjects is shown in the following table:

	HAV IgG/IgM Rapid Test		
Reference Test	Positive	Negative	Total
Positive	91	5	96
Negative	7	203	210
Total	98	208	306

Relative Sensitivity: 94.8% (95% CI: 88.3 - 98.3%)
Relative Specificity: 96.7% (95% CI: 93.3 - 98.6%)
Overall Agreement: 96.1% (95% CI: 93.3 - 98.0%)

4. Performance on Boston Biomedica Inc (BBI) Anti-HAV Seroconversion Panel

The performance of the HAV IgG/IgM Rapid Test was evaluated using the BBI Anti-HAV Seroconversion Panel (PHT903). The results are shown in the following table:

BBI Reference Panel: HAV ELISA		HAV IgG/IgM Rapid Test
Type	Number	Agreement
IgG HAV Positive	8	8 (100%)
IgG HAV Negative	2	2 (100%)
IgM HAV Positive	8	8 (100%)
IgM HAV Negative	2	2 (100%)

5. Positive Rate on the Random Clinical Specimens

990 random, clinical specimens were tested with the HAV IgG/IgM Rapid Test. The positive rate was 70.4% for IgG anti-HAV and 4.6% for IgM anti-HAV.

6. Cross-Reactivity

No false positive anti-HAV IgG and IgM results were observed on 4-10 specimens from the following disease states or special conditions, respectively:

HBV	HCV	HEV	HIV	hCG
Dengue	*H. pylori*	Malaria	TB	*T. pallidum*
Typhoid	ANA	HAMA	RF (up to 1,000 IU/mL)	

7. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the HAV IgG/IgM Rapid Test. Possible interference was studied by spiking these substances into negative, anti-HAV IgG positive and IgM positive specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the HAV IgG/IgM Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Salicylic acid	4.24 mmol/L
4. EDTA	3.4 µmol/L	9. Sodium citrate	3.4%
5. Glucose	55 mmol/L		

EXPECTED VALUES

Approximately 1.4 million clinical cases of hepatitis A occur worldwide annually[10]. Incidence rate is strongly related to socioeconomic indicators, access to safe drinking water, and vaccination. In less developed countries with poor sanitary and hygienic conditions, HAV is endemic and most people become infected in early childhood. Seroprevalence of anti-HAV is highest in some areas in Africa, Asia and Central and South America, where it can reach up to 100% in children[10]. In most developed regions, such as North America, Western Europe, Australia and Japan, anti-HAV prevalence in children can be as low as 10%[10].

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to HAV in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The HAV IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to HAV in human serum, plasma or whole blood. The intensity of the test line does not have linear correlation with the antibody titer in the specimen.
3. A negative or non-reactive test result does not preclude the possibility of exposure to or infection with HAV. A negative or non-reactive result can occur if the titer of HAV antibodies present in the specimen is below the level detectable by the assay or if HAV antibodies were not present during the stage of disease in which the sample was collected.
4. A negative result does not rule out an acute infection with HAV. Samples collected too early in the course of an infection may have levels of IgM that are below the limit of detection of this test.
5. Infection may progress rapidly. If the symptoms persist, even if the result from HAV IgG/IgM Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
6. Unusually high titers of heterophile antibodies or rheumatoid factor (≥1,000 IU/mL) may affect expected results.
7. The presence of anti-Hepatitis A IgG antibodies can be observed due to past infections and/or vaccination[11].
8. Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

REFERENCES

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Index of CE Symbols

Consult instructions for use	For *in vitro* diagnostic use only	Use by
Catalog #	Lot Number	Tests per kit
Store between 2-30°C	Authorized Representative	Do not reuse
Manufacturer	Date of manufacture	

Mfd. By: M/s. CTK Biotech, Inc.
13855 Stowe Drive, Poway, California 92064, USA (United States) having factory premises at M/s Beijing Genesee Biotech Inc., #36, Yanqi Donger Road, Huairou Yanqi Industrial Development Zone, Beijing, China

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

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English Version

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