OnSite® HAV IgM Rapid Test

REF R0090C CE

Instructions for Use

INTENDED USE

The *OnSite* HAV IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of IgM antibodies to hepatitis A virus (HAV) in human serum, plasma or whole blood. It is intended to be used by professionals as a preliminary test result to aid in the diagnosis of infection with HAV.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on 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

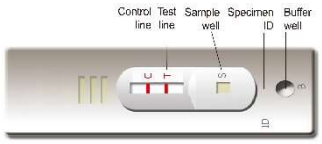
HAV, a positive-sense RNA virus, is a unique member of the family *Picornaviridae*[1]. Its transmission depends primarily on serial transmission from person-to-person by the fecal-oral route. Although hepatitis A is not ordinarily a sexually transmitted disease, the infection rate is high among men who have sex with men as a result of oral-anal contact[2,3].

The presence of specific anti-HAV IgM in blood samples suggests an acute or recent HAV infection[4-6]. 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 in most patients[7].

The *OnSite* HAV IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of anti-HAV IgM in serum, plasma or whole blood. It can be performed within 15 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The *OnSite* HAV IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing HAV antigen conjugated with colloidal gold (HAV Ag conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with mouse anti-human IgM, and the C line is pre-coated with a control line antibody.



When an adequate volume of test specimen and sample diluent are dispensed into the sample and buffer wells of the cassette, respectively, the specimen migrates by capillary action across the cassette. Anti-HAV IgM, if present in the specimen, will bind to the HAV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgM forming a colored T line, indicating an IgM anti-HAV positive test result. Absence of the T line suggests an anti-HAV IgM negative test result.

The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of control line antibodies regardless of any color development on the T line. If the C line does not develop, 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-R0090, 5 mL/bottle)
4. 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 test

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these instructions for use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch unless 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 the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimens 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 blood-borne 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 the patient specimens.
12. The test result should be read 15-20 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit 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 kit components to room temperature if stored cold. Thaw serum or plasma specimen if stored frozen. Blood specimen must be stored at 2-8°C and tested within 24 hours of collection. Mix all specimen well prior to performing the assay.

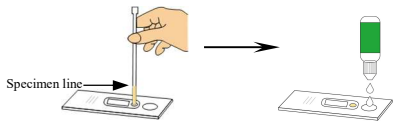
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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.***

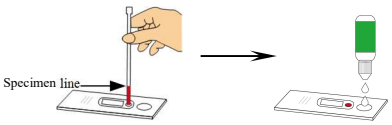
Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well (**S well**), making sure that there are no air bubbles.

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

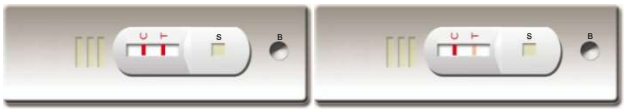
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

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 specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used 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

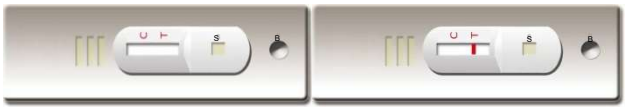
1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that no detectable anti-HAV IgM is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT:** If both the C and the T lines develop, the test indicates the presence of detectable anti-HAV IgM in the specimen. The result is positive or reactive.



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 5.*

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 306 specimens were collected from susceptible subjects and tested by the *OnSite* HAV IgM Rapid Test and by a leading commercial ELISA kit in Europe and other regions. Comparison for all subjects is shown in the following table:

	OnSite HAV IgM Rapid Test		
HAV IgM ELISA Test	**Positive**	**Negative**	**Total**
Positive	87	9	96
Negative	5	205	210
Total	92	214	306

Relative Sensitivity: 90.6% (95% CI: 82.9-95.6%),
Relative Specificity: 97.6% (95% CI: 94.5-99.2%),
Overall Agreement: 95.4% (95% CI: 92.4-97.5%).

2. **Cross Reactivity**

No false positive results were observed on 3-10 specimens from the following disease states or special conditions, respectively:

Dengue	HBV	HCV	HEV	HIV
Malaria	TB	*T. palladium*	Typhoid	ANA
RF (up to 1,000 IU/mL)				

3. **Interference**

The interference of chemicals commonly found in OTC and prescription medications and blood components on the performance of the *OnSite* HAV IgM Rapid Test was studied by spiking these substances into three levels of standard control: negative, weak positive, and strong positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *OnSite* HAV IgM Rapid Test.

List of potentially inferring 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. Human IgG	1,000 mg/dL
4. EDTA	3.4 µmol/L	9. Salicylic acid	4.34 mmol/L
5. Glucose	55 mmol/L	10. Sodium citrate	3.0%

4. **BBI Panel**

The BBI HAV seroconversion panel PHT903 was tested with the *OnSite* HAV IgM Rapid Test. The test results are presented in the table below.

BBI Panel PHT903	Abbott AxSYM HAV IgM S/co*	*OnSite* HAV IgM Rapid Test
01	0.1	Negative
02	0.1	Negative
03	4.8	Positive
04	4.8	Positive
05	4.8	Positive
06	4.1	Positive
07	2.0	Positive
08	1.4	Positive
09	1.2	Positive
10	1.3	Positive

*S/co ratios ≥ 1.0 considered reactive

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of anti-HAV IgM in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* HAV IgM Rapid Test is limited to the qualitative detection of anti-HAV IgM 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 anti-HAV IgM present in the specimen is below the level detectable by the assay or if anti-HAV IgM was not present during the stage of disease in which the sample was collected.
4. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* HAV IgM Rapid Test is negative or non-reactive, it is recommended to re-test the patient a few days later or test with an alternative test method.
5. Unusually high titers of heterophile antibodies or rheumatoid factor (≥1,000 IU/mL) may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Minor P, Francki RIB, Fauquet CM, et al. Classification and nomenclature of viruses. Fifth Report of the International Committee on Taxonomy of Viruses 1991. 320-326.
2. Keeffe EB. Clinical approach to viral hepatitis in homosexual men. Med Clin North Am 1986. 70(3):567-586.
3. Ballesteros J, Dal-Re R, Gonzalez A, et al. Are homosexual males a risk group for hepatitis A infection in intermediate endemicity areas? Epidemiol Infect 1996. 117(1):145-148.
4. Bradley DW, Maynard JE, Hindman SH, et al. Serodiagnosis of viral hepatitis A: detection of acute-phase immunoglobulin M anti-hepatitis A virus by radioimmunoassay. J Clin Microbiol 1977. 5(5):521-530.
5. Decker RH, Kosakowski SM, Vanderbilt AS, et al. (1981). Diagnosis of acute hepatitis A by HAVAB-M, a direct radioimmunoassay for IgM anti-HAV. Am J Clin Path 1981. 76(2):140-147.
6. Locarnini SA, Ferris AA, Lehmann NI, et al. The antibody response following hepatitis A infection. Intervirology 1977. 8(5):309-318.
7. Skinhøj P, Mikkelsen F, & Hollinger FB. Hepatitis A in Greenland: importance of specific antibody testing in epidemiologic surveillance. Am J Epidemiol 1997. 105(2):140-147.

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	

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

For Export Only. Not For Resale in the USA.

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

PI-R0090C Rev. H2.0
Date released: 2020-11-12
English Version