OnSite™ HAV IgM Rapid Test

REF R0090C CE

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 as a screening test by professionals and as an aid in the diagnosis of infection with HAV. Any reactive result with the *OnSite* HAV IgM Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

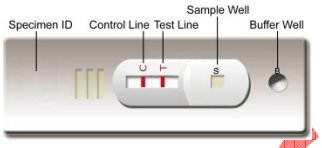
SUMMARY AND EXPLANATION OF THE TEST

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 IgM anti-HAV in blood samples suggests an acute or recent HAV infection[4-6]. IgM anti-HAV 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 IgM anti-HAV in serum, plasma or whole blood. The *OnSite* HAV IgM Rapid Test 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 burgundy 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. IgM anti-HAV, 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 burgundy colored T line, indicating an IgM anti-HAV positive test result. Absence of the T line suggests an IgM anti-HAV negative test result.

The test contains an internal control (C line) which should exhibit a burgundy 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-R0090C, 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 test

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 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 within 15 minutes after a specimen is applied to the sample well of the device. Reading the result after 20 minutes may give erroneous results.
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 over 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. If not tested immediately, 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.

Blood

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). 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.

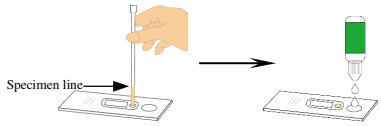
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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.***

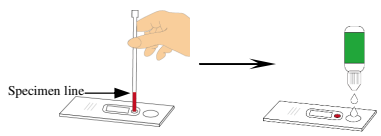
Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well (**S well**), making sure that there are no air bubbles.

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 result in 15 minutes. Positive results may be visible in as soon as 1 minute.

Do not read the result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

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. 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 is developed, the test indicates that no detectable IgM anti-HAV is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT**
If both the C and the T lines are developed, the test indicates the presence of detectable IgM anti-HAV 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.*

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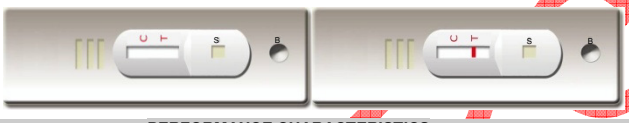
3. **INVALID**
If no C line is developed, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**
A total of 306 patient samples from susceptible subjects were 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%, Relative Specificity: 97.6%, Overall Agreement: 95.4%

2. **Cross Reactivity**
Specimens from various disease stages were tested for cross-reactivity with the *OnSite* HAV IgM Rapid Test according to the standard procedure. The results are presented below.

Specimens	**Sample size**	***OnSite* HAV IgM Rapid Test**
Typhi positive serum	10	Negative
Dengue positive serum	8	Negative
HBsAg positive serum	10	Negative
HCV positive serum	10	Negative
HEV positive serum	10	Negative
HIV positive serum	10	Negative
Malaria positive serum	10	Negative
RF (≤1,000IU/mL)	3	Negative
Syphilis positive serum	10	Negative
TB positive serum	10	Negative
ANA	6	Negative

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 are presented in the table below. Collectively, at the concentrations tested, no interference was observed.

Note: -: Negative; +: Weak Positive; +++: Strong Positive

Potential interfering substances spiked	***OnSite* HAV IgM Rapid Test**		
	Negative	**Weak positive**	**Strong positive**
Control	-	+	+++
Albumin 60 g/L	-	+	+++
Bilirubin 20 mg/dL	-	+	+++
Creatinine 442 µmol/L	-	+	+++
EDTA 3.4 µmol/L	-	+	+++
Glucose 55 mmol/L	-	+	+++
Hemoglobin 2 g/L	-	+	+++
Heparin 3,000 U/L	-	+	+++
Human IgG 1,000 mg/dL	-	+	+++
Salicylic acid 4.34 mmol/L	-	+	+++
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 IgM anti-HAV 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 IgM anti-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 IgM anti-HAV present in the specimen is below the level detectable by the assay or if IgM anti-HAV 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.
10110 Mesa Rim Road
San Diego, CA 92121, 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. H
Effective date: 2015-03-05
English Version