OnSite® HEV IgM Rapid Test

REF R0095C CE

Instructions for Use

INTENDED USE

The *OnSite* HEV IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of anti-hepatitis E virus (HEV) IgM in human serum or plasma. It is intended to be used as a screening test by professionals and provides a preliminary test result to aid in the diagnosis of infection with HEV.

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

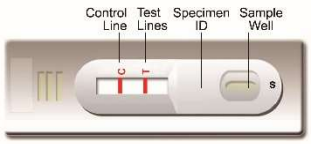
SUMMARY AND EXPLANATION OF THE TEST

Hepatitis E, a major form of enterically transmitted hepatitis, is widespread in many developing countries but is currently considered an emerging threat to other parts of the world. HEV is a non-enveloped, positive-sense, single-stranded RNA virus[1,2]. It is currently classified within the family *Caliciviridae*. It is mainly transmitted through fecal-oral route. At least four major genotypes of HEV have been recognized[3]: genotypes 1 and 2 are restricted to humans while genotypes 3 and 4 can infect both humans and animals. Antibody responses peak at about one month after initial infection. Antiviral IgM is detected in >90% of patients and persists for 3 months. Anti-HEV IgM is also a well-established marker of recent infection[4] and is the most convenient one for diagnosis[5,6].

Reliable techniques for anti-HEV IgM detection such as immunofluorescence and immune electron microscopy (IEM) have been developed. However, these techniques require labor-intensive procedures that are not available to many laboratories. The *OnSite* HEV IgM Rapid Test is designed to detect anti-HEV IgM in human serum or plasma. It can be performed within 15 minutes by minimally skilled personnel without laboratory equipment.

TEST PRINCIPLE

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



When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. Anti-HEV IgM if present in the specimen will bind to the HEV conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a colored T line, indicating a HEV IgM positive test result and suggesting an acute infection.

Absence of the test line suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control line antibodies regardless of color development on the T line. Otherwise, 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. 10 µL Capillary tubes
3. Sample diluent (REF SB-R0095, 5 mL/bottle)
4. Instructions for Use

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer

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.
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 patient specimens.
12. The testing results should be read 15 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 15-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 device 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 temperature 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.

ASSAY PROCEDURE

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

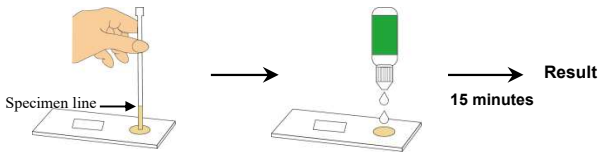
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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

Step 4: Fill the capillary tube with the specimen not to exceed the specimen line as indicated in the illustration below.

Holding the capillary tube vertically, dispense 10 µL of specimen into the sample well making sure that there are no air bubbles.

Then, immediately add 2 drops (about 70-100 µL) of sample diluent holding the bottle vertically.



10 µL Serum or Plasma **2 drops of sample diluent**

Step 5: Set up the timer.

Step 6: Results can be read at 15 minutes. Positive results can be visible in as short as 1 minute. However, negative results must be confirmed at the end of 15 minutes only. ***Any results interpreted outside of the 15-minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local laws governing the disposal of devices.***

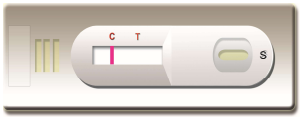
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the whole procedure and repeat 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 testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature during storage of the kit 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 IgM anti-HEV is present in the specimen. The result is negative or non-reactive.



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

Samples with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

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

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 1060 samples were collected from susceptible subjects and tested by the *OnSite* HEV IgM Rapid Test and by a commercial ELISA test. Comparison for all subjects is shown in the following table:

	OnSite HEV IgM Rapid Test		
HEV IgM ELISA	**Positive**	**Negative**	**Total**
Positive	314	6	320
Negative	6	734	740
Total	320	740	1060

Relative Sensitivity: 98.1% (95% CI: 96.0-99.3%),
Relative Specificity: 99.2% (95% CI: 98.2-99.7%),
Overall Agreement: 98.9% (95% CI: 98.0-99.4%).

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-HEV IgM in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* HEV IgM Rapid Test is limited to the qualitative detection of anti-HEV IgM in human serum or plasma. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-HEV IgM. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with HEV.
4. A negative or non-reactive result can occur if the quantity of the anti-HEV IgM present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. Infection may progress rapidly. If the symptoms persist and the result from *OnSite* HEV IgM Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method such as ELISA or PCR.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Tong MJ, Roue R, Nahor M et al: Clinical aspects of hepatitis E and hepatitis A: a comparison . In: Enterically transmitted hepatitis viruses (eds Buisson Y, Coursaget P and Kane M), 1-10 (La Simarre, Tours, 1996)
2. Labrique AB, Thomas DL, Stoszek SK, et al: Hepatitis E: an emerging infectious disease . Epidemiol. Rev. 1999; 21: 162-179
3. Thomas HC, Lemon SL, and Zuckerman AJ, Viral hepatitis, 3d ed, Wiley-Blackwell, New York, Sep 5, 2005, p612.
4. Rose NR, Hamilton RG, and Detrick B. Manual of clinical laboratory immunology, 6th, ed ASM Press, Washington DC, 2002, p710.
5. Ghabrah TM, Tsarev S, Yarbough PO, et al: Comparison of tests for antibody to hepatitis E virus. J. Med. Virol. 1998; 55:134-137.
6. Yu C, Engle RE and Bryan JP: Detection of immunoglobulin M antibodies by class capture immunoassay. Clinical and diagnostic laboratory immunology, 2003;10:579-586.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative	2	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

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R0095C Rev. E2.0
Date released: 2020-11-12
English version

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