HEV IgM Rapid Test- (Serum / Plasma)

BIOGENIX INC. PVT. LTD.

IVD **In Vitro Diagnostic**

INTENDED USE

The HEV IgM Cassette Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of IgM antibodies to Hepatitis E virus (HEV) in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HEV. Any reactive specimen with the HEV IgM Cassette Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

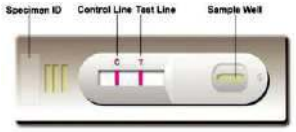
SUMMARY AND EXPLANATION OF THE TEST

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 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 HEV IgM Cassette Rapid Test is designed to detect anti-HEV IgM in less than 15 minutes by untrained or minimally skilled personnel without cumbersome laboratory equipment.

TEST PRINCIPLE

The HEV IgM Cassette Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing HEV antigens conjugated with colloidal gold (HEV conjugates) and rabbit IgG-gold conjugates, 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 goat anti-rabbit IgG 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 burgundy colored T line, indicating a HEV IgM positive test result.

Absence of the test line suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugate 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. Sample diluent (1 bottle, 5 ml)
3. 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. Capillary tubes (10 µL)

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 gives 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°C-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 hemolized 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 biohazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. 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°C-30°C. Do not expose the kit over 30°C. Do not freeze the kit. If stored at 2°C-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.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
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 veinpuncture.
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. Store specimens at 2°C-8°C if not tested immediately.

Store specimens at 2°C-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.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
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.

10µL specimen 2 drops of sample diluent

Step 5: Set up the timer.
Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 15 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 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. 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 used during storage of the kit falls outside of 2-30℃.
   e. The temperature of the test area falls outside of 15 -30℃.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

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INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT: If only the C line is developed, 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 are developed, the test indicates the presence of IgM anti-HEV 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 positive determination is made.

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

PERFORMANCE CHARACTERISTICS

Clinical Performance

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

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

Relative Sensitivity: 98.1%, Relative Specificity: 99.2 %, Overall Agreement: 98.9%

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 HEV IgM Cassette 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. If the symptoms persist and the result from HEV IgM Cassette Rapid Test is negative or non-reactive, it is recommended to re-sample the patient few days later or 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.

WASTE MANAGEMENT OR DISPOSABLE:

The contents of RDTs can be divided into :

Infectious waste:

- sharps (lancets, needles, scalpel blades)
- blood collection devices (tubes, straws, and loops); gloves; swabs; and cotton
- used cassettes.

Non-infectious waste (Recyclable):

- packaging materials, desiccant, buffer, and unused or unusable RDTs.

*****You must collect and dispose each type of waste in separate containers as per your waste management policies.***

Index of CE Symbols

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

Mfg. By: BIOGENIX INC. PVT. LTD.
Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road,Lucknow - 226008 (U.P.), India
Email: biogenix2007@yahoo.com, info@biogenixinc.com
Website: www.biogenixinc.com
Customer care no:+919140971443

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