HEV IgG/IgM Rapid Test - Cassette ( Serum/Plasma/Whole Blood)

**INTENDED USE**

The OnSite HEV IgG/IgM Rapid Test is a lateral flow chromatographic immunassay for the simultaneous detection and differentiation of IgG and IgM antibodies to Hepatitis E virus (HEV) in human serum, plasma or whole blood. This test is intended for investigational use only; not for use in diagnostic procedures.

**SUMMARY AND EXPLANATION OF THE TEST**

Hepatitis E Virus (HEV) is a non-enveloped, positive-sense, single-stranded RNA virus, classified within the family of Hepeviridae. HEV is a major cause of enterically transmitted hepatitis, that is widespread in many developing countries, and currently considered an emerging threat to other parts of the world[1,2]. The disease is mainly transmitted through the fecal-oral route. After an incubation period of 2-8 weeks, approximately 25% of patients develop symptoms, including flu-like myalgia, arthralgia, anorexia, hepatomegaly, fever, weakness, and vomiting, and sometimes jaundice, itching, pale stools, and darkened urine. HEV infection can lead to more severe liver disease in pregnant women or patients with underlying chronic liver diseases[3].

The antibody response to HEV peak about one month after initial infection. Anti-HEV IgM is detected within 2 weeks in >90% of patients with acute infection and persists for up to 5 months. Anti-HEV IgG antibodies are detectable shortly after the appearance of anti-HEV IgM, and persist for >1 to 14 years post infection[3].

The OnSite HEV 1+2 IgG/IgM Rapid Test allows detection and differentiation of IgG and IgM antibodies to HEV in serum, plasma or whole blood in 15 minutes. The test can be performed by minimally skilled personnel without the use of laboratory equipment.

**TEST PRINCIPLE**

The OnSite HEV IgG/IgM Rapid Test is a lateral flow chromatographic immunassay. The test cassette consists of: 1) a burgundy 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 two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgM for detection of IgM anti-HEV, and the C line is pre-coated with a control antibody conjugated with colloidal gold. When an adequate volume of test specimen is applied to the sample well of the cassette, the test specimen migrates by capillary action across the cassette. IgM anti-HEV, if present in the specimen, will bind to the HEV conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgM forming a burgundy-colored M line, indicating a HEV IgM positive test result. IgG anti-HEV, if present in the specimen, will bind to the HEV conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a burgundy-colored G line, indicating a HEV IgG positive test result. Absence of any T 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. 10 µL capillary pipettes
3. Sample diluent (REF SB-R0096, 5 mL/bottle)
4. One package insert (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
2. Lancing device (for whole blood testing)

**WARNINGS AND PRECAUTIONS**

For Investigational Use Only

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.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use components from another test kit or from another test 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 HEV, 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, negative and positive controls, if provided, in the same manner as patient specimens.
12. The test results should be read 15 minutes after a specimen is applied to the sample well 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, e.g. an electric fan or strong air conditioning.

**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 ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to testing specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The storage temperature of the kits falls outside of 2-8°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.

**SPECIMEN COLLECTION AND HANDLING**

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

**Plasma**

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

**Serum**

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

**Blood**

Whole blood can be obtained by either finger tip puncture or venipuncture. Collect a blood specimen into an EDTA, citrate or heparin-coated collection tube (lavender, blue or green top, respectively, for Vacutainer® tubes). Do not use hemolyzed blood for testing.

Whole blood specimens should be refrigerated (2-8°C) if not tested immediately. 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 test device. Place the test device on a clean flat surface.

Step 3: Label the device with the specimen ID number.

Step 4: Using a squeezing motion, fill the capillary pipette with the serum, plasma or whole blood. Do not exceed the specimen line (see following image). The volume of the specimen is approximately 10 µL. For maximum precision, transfer the specimen using a pipette capable of delivering 10 µL volumes.

**WARNING**

Hold the capillary pipette vertically, dispense the entire specimen into the center of the specimen well (S well), making sure that there are no air bubbles.

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

**RESULT**

10 µL serum/plasma
2 drops of sample diluent

Result

10 µL whole blood
2 drops of sample diluent

15 minutes

**REAGENT PREPARATION AND STORAGE INSTRUCTIONS**

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C or 15-30°C and use within the time periods as shown on the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures over 30°C.

**OnSite REF R0096C**
1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that no detectable IgG or IgM anti-HEV is present 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 IgG anti-HEV; the result is HEV IgG positive or reactive.

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

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

3. **INVALID:** If no C line develops, the assay 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. **Clinical Performance: IgM**
   A total of 146 patient samples from susceptible subjects were tested by the OnSite HEV IgG/IgM Rapid Test and by a commercially available HEV IgM EIA. The results are shown in the following table:

<table>
<thead>
<tr>
<th>OnSite HEV IgG/IgM Rapid Test</th>
<th>HEV IgM EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5</td>
<td>136</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>135</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>271</td>
<td>292</td>
<td></td>
</tr>
</tbody>
</table>

   Relative Sensitivity: 100%, Relative Specificity: 95.7%, Overall Agreement: 95.9%

2. **Clinical Performance: IgG**
   A total of 146 patient samples from susceptible subjects were tested by the OnSite HEV IgG/IgM Rapid Test and by a commercially available HEV IgG EIA. The results are shown in the following table:

<table>
<thead>
<tr>
<th>OnSite HEV IgG/IgM Rapid Test</th>
<th>HEV IgG EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>57</td>
<td>10</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>78</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>88</td>
<td>148</td>
<td></td>
</tr>
</tbody>
</table>

   Relative Sensitivity: 85.1%, Relative Specificity: 96.2%, Overall Agreement: 91.1%

**EXPECTED VALUES**

HEV exhibits distinguishable epidemiological patterns in endemic regions compared to nonendemic areas. Endemic countries have exhibited an overall HEV prevalence of 25% of all non-A, non-B acute hepatitis cases, and while the anti-HEV IgG prevalence among healthy blood donors may be as high as 45% in some hyperendemic countries, reports from industrialized countries show prevalence ranging from 1-4%. Outbreaks have been observed in China, the Indian subcontinent, southeast and central Asia, the Middle East, and the northern and western parts of Africa. Outbreaks usually affect several hundred to several thousand persons, with overall attack rates ranging from 1-15%, and males usually outnumbering females.

**TEST LIMITATIONS**

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to HEV virus in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.

2. The OnSite HEV IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to HEV virus 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 HEV. A negative or non-reactive result can occur if the titer of HEV antibody present in the specimen is below the level detectable by the assay or if HEV antibody was not present during the stage of disease in which the sample was collected.

**REFERENCES**


