The OnSite HBV 5-Parameter Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb 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 HBV. Any reactive specimen with the OnSite HBV 5-Parameter Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

**SUMMARY AND EXPLANATION OF THE TEST**

Hepatitis virus B (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have extant for several millennia. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Western countries) to 20% (Asia, Africa)1.

HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase2, the core antigen (HBcAg)3, and the e antigen (HBeAg)4. The HBcAg is enclosed in a coat that contains lipid, protein and carbohydrate and expresses an antigen termed hepatitis B surface antigen (HBsAg)5.

HBsAg is the first marker to appear in the blood in acute hepatitis B, being detected 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms. Simultaneously with or shortly after the disappearance of HBsAg, antibody to HBsAg (anti-HBs) is found in the blood. Its appearance heralds complete recovery, and its presence provides lifelong immunity6.

Antibody to HBcAg (anti-HBc) appears shortly after HBsAg, roughly at the time that serum ALT begins to rise. Anti-HBc also remains elevated for life and is a useful marker of previous HBV infection. HBsAg itself does not circulate freely in the serum of such infected persons7-8.

HBeAg is seen in the blood before the onset of clinical disease and after the appearance of HBsAg. HBeAg generally disappears within about 2 weeks, while HBsAg is still present. Anti-HBe appears shortly after the disappearance of the antigen and is detectable for up to 2 years or more after resolution of the hepatitis. The presence of HBeAg in the serum correlates with a period of intense viral replication and hence, maximal infectivity of the patient9-10.

Clinically, HBsAg, HBsAb, HBeAg, HBeAb and HBcAb are the important markers in the diagnosis of HBV infection.

The OnSite HBV 5-Parameter Rapid Test is a 5-panel rapid test that can detect HBsAg, HBsAb, HBeAg, HBeAb and HBcAb simultaneously by untrained or minimally skilled personnel, without laboratory equipment.

**TEST PRINCIPLE**

The OnSite HBV 5-Parameter Rapid Test is lateral flow chromatographic immunoassay consisting of 5 test panel strips assembled in one cassette. Each strip of the panel member is composed of a sample pad, colloidal gold conjugate pad, nitrocellulose membrane (NC membrane) strip pre-coated with control band (C band) and test band (T band), and absorbent pad.

The HBsAg strip is an antibody based sandwich immunoassay. The conjugate pad contains polyclonal anti-HBsAg antibodies conjugated with colloidal gold and the NC membrane is pre-coated with a monoclonal anti-HBsAg. When an adequate volume of test specimen is applied into the sample well of the strip, the test specimen migrates by capillary action across the test strip. HBsAg if present in the specimen will bind to the anti-HBsAg gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-HBsAg antibody, forming a burgundy colored T band, indicating a HBsAg positive test result. Absence of the T band suggests a negative result.

The HBeAg strip is also an antibody based sandwich immunoassay. The test utilizes a pair of anti-HBeAg antibodies to detect HBeAg in the test specimen (see the HBeAg principle for explanation). A burgundy colored T band indicates a HBeAg positive test result and absence of the T band suggests a negative result.

The HBsAb strip is an antigen based sandwich immunoassay. The conjugated pad contains HBsAg conjugated with colloidal gold and the NC membrane is pre-coated with un-conjugated HBsAg. HBsAb if present in the patient specimen will bind to the HBsAg gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated HBsAb, forming a burgundy colored T band, indicating a HBsAb positive test result. Absence of the T band suggests a negative result.

The HBeAb strip is a competitive immunomassay. The conjugated pad contains anti-HBe antibody conjugated with colloidal gold (HBeAb conjugates) and the NC membrane is pre-coated with HBeAb. If no HBeAb or its level in the specimen is below the test sensitivity, the HBeAb conjugates will have enough binding sites to bind to the HBeAb coated on the NC membrane, therefore forming HBeAb conjugates-HBeAg immunocomplex and leading to a burgundy colored T band, indicating a negative result. If the levels of HBeAb in the specimen is at or higher than the test sensitivity, it will bind to the HBeAg on the NC membrane, competing the binding of the HBeAb conjugates to the HBeAg. Therefore, absence of the T band indicates a positive test result.

The HBcAb strip is also a competitive immunomassay. The conjugated pad contains anti-HBc antibody conjugated with colloidal gold and the NC membrane is pre-coated with HBcAb (See the HBcAb principle for the explanation). A burgundy colored T band suggests a negative result and absence of the T band indicates a positive test result.

**REAGENT AND MATERIALS PROVIDED**

1. Each kit contains 25 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One pipette dropper.
   c. One desiccant.
2. One package insert (instruction for use).

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Clock or Timer

**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 specimen 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 hazardous waste.
11. Handle the Negative and Positive Control 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 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. The positive and negative controls should be kept at 2°C-8°C. 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. Do not freeze the kit or expose the kit over 30°C.

**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 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. Store specimens at 2°C-8°C if not tested immediately.

Store specimens at 2°C-8°C 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 on result interpretation.

**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 pipette dropper with the specimen. Holding the dropper vertically, dispense 2-3 drops (about 60-90 µL) of specimen into each of the sample well making sure that there are no air bubbles.

**Step 5:** Set up 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**

Using individual the OnSite HBV 5-Parameter Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C -30°C.
5. The temperature of the test area falls outside of 15°C -30°C.

Expected results are as follows:

<table>
<thead>
<tr>
<th>Strips</th>
<th>C Band</th>
<th>T Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbcAb</td>
<td>Visible</td>
<td>Visible</td>
</tr>
<tr>
<td>HbsAb</td>
<td>Visible</td>
<td>No band</td>
</tr>
<tr>
<td>HbsAg</td>
<td>Visible</td>
<td>No band</td>
</tr>
<tr>
<td>HbeAb</td>
<td>Visible</td>
<td>No band</td>
</tr>
<tr>
<td>HbeAg</td>
<td>Visible</td>
<td>No band</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF ASSAY RESULT**

1. **NEGATIVE RESULT:** If only the C band is developed on the HbsAg, HbsAb, HbeAg strip, or both C and T bands are developed on either the HbsAb or the HbcAb strip, the test indicates a negative result on the parameter being tested.

<table>
<thead>
<tr>
<th>Strips</th>
<th>C Band</th>
<th>T Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbcAb</td>
<td>Visible</td>
<td>Visible</td>
</tr>
<tr>
<td>HbsAb</td>
<td>Visible</td>
<td>No band</td>
</tr>
<tr>
<td>HbsAg</td>
<td>Visible</td>
<td>No band</td>
</tr>
</tbody>
</table>

2. **POSITIVE RESULT:** If both C and T bands are developed on the HbsAg, or HbsAb, or HbeAg strip or only the C band is developed on the HbeAb or HbcAb strip, the test indicates presence of the parameter being tested. The result is positive.

<table>
<thead>
<tr>
<th>Strips</th>
<th>C Band</th>
<th>T Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbcAb</td>
<td>Visible</td>
<td>No band</td>
</tr>
<tr>
<td>HbsAb</td>
<td>Visible</td>
<td>Visible</td>
</tr>
<tr>
<td>HbsAg</td>
<td>Visible</td>
<td>Visible</td>
</tr>
</tbody>
</table>

**Note:** Add 1 drop of Saline or Phosphate-Saline buffer (common buffers used in clinic, not provided in the kit) into the sample well if flow migration is not observed in 30 seconds in the result window, which could occur with a highly viscous specimen.

**Limitations of Test**

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

<table>
<thead>
<tr>
<th>Strips</th>
<th>C Band</th>
<th>T Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbcAb</td>
<td>No band</td>
<td>regardless</td>
</tr>
<tr>
<td>HbsAb</td>
<td>No band</td>
<td>regardless</td>
</tr>
<tr>
<td>HbsAg</td>
<td>No band</td>
<td>regardless</td>
</tr>
<tr>
<td>HbeAg</td>
<td>No band</td>
<td>regardless</td>
</tr>
</tbody>
</table>

**Note:** Invalid of a particular parameter does not affect the result interpretation on the other valid test parameters.

**PERFORMANCE CHARACTERISTICS**

**Clinical Performance**

Please see the Performance Characteristics from the individual test insert.

**REFERENCES**