**INTENDED USE**

The OnSite HBsAg Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma at the level equal or higher than 2 ng/ml. It is intended to be used as a screening test and as an aid in the diagnosis of infection with Hepatitis B virus (HBV). Any reactive specimen with the OnSite HBsAg 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 been 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 core of HBV is enclosed in a coat that contains lipid, protein, and carbohydrates 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. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups.

The OnSite HBsAg Rapid Test detects HBsAg in serum or plasma in less than 15 minutes by untrained or minimally skilled personnel, without laboratory equipment.

**TEST PRINCIPLE**

The OnSite HBsAg Rapid Test is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing mouse anti-HBsAg antibody conjugated with colloidal gold (HBsAb conjugates) 2) a ninhydrin membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with non-conjugated anti-HBsAg antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.

When an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. HBsAg if present in the specimen will bind to the HBsAb conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-HBsAg antibody, forming a burgundy colored T band, indicating an HBsAg positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG / HBsAb-gold conjugate regardless of the presence of colored T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

**REAGENT PREPARATION AND STORAGE INSTRUCTIONS**

All reagents are ready to use as supplied. Store unused test device 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 vein puncture.
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 vein puncture.
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 to 8°C if not tested immediately.

Store specimens at 2°C to 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:** Collect at least 150-200 µL or 3-4 drops of serum or plasma in a sample container.

**Step 3:** When ready to test, open the pouch at the notch and remove the test strip.

**Step 4:** Dip the strip into the specimen for at least 10 seconds. Don’t allow the specimen reach above the level indicated by the arrows on the strip.

Meanwhile, set up timer.

**Step 5:** Remove the strip from the specimen, and place it on a flat, dry surface.

**Step 6:** Read the test result in 15 minutes. Positive result could be visible as short as 1 minute.

Don’t read results after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

**QUALITY CONTROL**

Using individual OnSite HBsAg Rapid Test strips as described in the Assay Procedure above, run 1 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:

**Negative Control**
Only the C band shows color development. The T band shows no color development.

**Positive Control**
Both C and T bands show color development.

The appearance of any burgundy color in the T band, regardless of intensity, must be considered as presence of the band.

### INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is developed, the test indicates that the level of HBsAg in the specimen is undetectable (lower than 2 ng/mL). The result is negative.

2. **POSITIVE RESULT**: If both C and T bands are developed, the test indicates that the specimen contains HBsAg at the level equal or higher than 2 ng/mL. The result is positive.

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

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

### PERFORMANCE CHARACTERISTICS

**Clinical Performance**
A total of 500 samples from susceptible subjects were tested by the OnSite HBsAg Rapid Test and by HBsAg ELISA kit with the test sensitivity at 0.5 ng/mL. Comparison for all subjects is showed in the following table.

<table>
<thead>
<tr>
<th>OnSite HBsAg Rapid Test</th>
<th>HBsAg ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>48</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HBsAg ELISA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>48</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>452</td>
<td>500</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 96%, Relative Specificity: 100%. Overall Agreement: 99.6%

### LIMITATIONS OF TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HBsAg in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The OnSite HBsAg Rapid Test is limited to the qualitative detection of HBsAg in human serum or plasma. The intensity of the test band does not have linear correlation with HBsAg titer in the specimen.
3. A negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (lower than 2 ng/mL), or the HBsAg that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.