OnSite® HBsAg Rapid Test

REF R0040S

Instructions for Use

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 a detection sensitivity level equal to or higher than 1 ng/mL. It is intended to be used by healthcare professionals to aid in the diagnosis of infection with hepatitis B virus (HBV).

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of 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

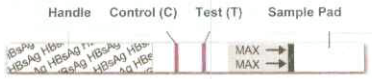
Hepatitis virus B (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have been in existence for several millennia. It is estimated that there are 300 million chronic carriers of 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 polymerase[2], the core antigen (HBcAg)[3] and the e antigen (HBeAg)[4]. The core of HBV is enclosed in a coat that contains lipid, carbohydrate and protein including an antigen termed hepatitis B surface antigen (HBsAg)[3].

HBsAg is the first marker to appear in the blood in acute hepatitis B, detectable 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, 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 15 minutes and can be performed by untrained or minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



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

When an adequate volume of test specimen is dispensed onto 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 HBsAg Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated non-conjugated HBsAg antibody forming a colored T line, indicating a HBsAg positive test result. Absence of the T 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 antibodies regardless of color development on the T line. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed pouches containing:
   a. One dip strip device
   b. One desiccant
2. Instructions for Use

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

3. Positive Control
4. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container for holding test specimen

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions 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 test materials to room temperature (15-30°C) before use.
5. Do not use components from 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 test result should be read 15-20 minutes after a specimen is applied to the sample well of the device. Reading the test result after 15-20 minutes should be considered invalid and must be repeated.
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 2-30°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 temperatures 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 thawed, mix the specimen well prior to assay.
Step 2: Collect at least 150-200 µL or 3-4 drops of serum or plasma in a sample container.
Step 3: Take the desired quantity of sealed pouches from the box. When ready to test, open the pouch at the notch and remove the test strip.
Step 4: Dip test strip into the specimen for at least 10 seconds. Do not allow the specimen to reach above the "MAX" level indicated by the arrows on the strip.
Step 5: Remove the strip from the specimen, and place on a flat, dry surface. Set up the timer.
Step 6: Read results at 15 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, any results interpreted outside 15-20 minutes should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.***

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen. If the C line does not develop, review the entire procedure and repeat the 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 test kits is used.
   d. The temperature used during storage of the kits 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 the level of HBsAg in the specimen is undetectable (lower than 1 ng/mL). The result is negative or non-reactive.

2. **POSITIVE RESULT**: If both the C and the T lines develop, the test indicates that the specimen contains HBsAg at a level equal to or higher than 1 ng/mL. The result is positive or reactive.

Specimens with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis decision 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 560 specimens were collected from susceptible subjects and tested with the *OnSite* HBsAg Rapid Test and with a commercial HBsAg ELISA kit with a test sensitivity of 0.5 ng/mL. Comparison for all subjects is shown in the following table:

	OnSite HBsAg Rapid Test		
HBsAg ELISA	**Positive**	**Negative**	**Total**
Positive	97	0	97
Negative	0	463	463
Total	97	463	560

Relative Sensitivity: 100% (95% CI: 97.3% - 100%)
Relative Specificity: 100% (95% CI: 99.4% - 100%)
Overall Agreement: 100% (95% CI: 99.5% - 100%)

2. **Cross-Reactivity**

No false positive results were observed on 3-10 specimens from the following disease states or special conditions, respectively:

Dengue	HAV	HCV	HIV	*H. pylori*
Syphilis	TB	ANA	HAMA	RF (up to 2,500 IU/mL)

3. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* HBsAg Rapid Test. This was studied by spiking these substances into three levels of HBsAg standard controls. The results are presented in the following table and demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *OnSite* HBsAg Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	50 g/L	6. EDTA	3.4 µmol/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Human IgG	1,000 mg/dL
4. Glucose	55 mmol/L	9. Sodium citrate	3.8%
5. Salicylic acid	4.34 mmol/L		

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of HBsAg in serum or plasma from individual subjects. Failure to follow the procedure may lead to 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 line does not have a linear correlation with the HBsAg titer in the specimen.
3. A non-reactive test result does not preclude the possibility of exposure to or infection with HBV.
4. A non-reactive result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (lower than 1 ng/ml) or the HBsAg that is detected was not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptoms persist when the result from *OnSite* HBsAg Rapid Test is non-reactive, it is recommended to re-sample the patient a few days later or to test with an alternative test method.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Emanuel Rubin and John Farber. The liver and biliary system. Acute viral hepatitis P 721-729. Rubin E, Farber JL ed. Pathology 2nd ed. 1994. J.B. Lippincott, Philadelphia
2. Kaplan PM, Greenman RL, Gerin JL, *et al.* DNA polymerase associated with human hepatitis B antigen. J Virol. 1973 12(5):995-1005.
3. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet. 1970;1(7649):695-8.
4. Magnius LO, Espmark A. A new antigen complex co-occurring with Australia antigen. Acta Pathol Microbiol Scand [B] Microbiol Immunol. 1972;80(2):335-7.

Index of 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		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

PI-R0040S Rev. G3.0
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English version

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