CTK BIOTECH SIMPLIFYING DIAGNOSTICS

REF

Catalog Number R0049C

IVD

In vitro Diagnostic

INTENDED USE

The *OnSite* HBV-5 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 Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (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 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, protein and carbohydrate and expresses an antigen termed hepatitis B surface antigen (HBsAg)[3].

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 (HBsAb) is found in the blood. Its appearance heralds complete recovery, and its presence provides lifelong immunity[1,4].

Antibody to HBcAg (HBcAb) appears shortly after HBsAg, roughly at the time that serum ALT levels begin to rise. HBcAb also remains elevated for life and is a useful marker of previous HBV infection. HBcAg itself does not circulate freely in the serum of such infected persons[1,4].

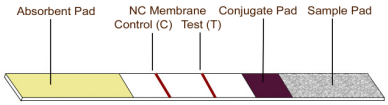
HBeAg is seen in the blood before the onset of clinical disease and after the appearance of HBsAg. HBeAg generally disappears within about 2 weeks while HBsAg is still present. HBeAb appears shortly after the disappearance of the antigen and is detectable for up to 2 years or more after resolution of the hepatitis infection. The presence of HBeAg in the serum correlates with a period of intense viral replication and hence, maximal infectivity of the patient[1,4].

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

The *OnSite* HBV-5 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 Rapid Test is lateral flow chromatographic immunoassay consisting of 5 test panel strips assembled in one cassette. Each strip of the panel is composed of a sample pad, colloidal gold conjugate pad, nitrocellulose membrane (NC membrane) strip pre-coated with a control line (C line) and test line (T line), 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 line, indicating a HBsAg positive test result. Absence of the T line 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 HBsAg principle for explanation). A burgundy colored T line indicates a HBeAg positive test result and absence of the T line 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 HBsAg forming a burgundy colored T line, indicating a HBsAb positive test result. Absence of the T line suggests a negative result.

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

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

All of the panel strips have an internal quality control system consisting of a mouse IgG antibody conjugated with colloidal gold and an NC membrane pre-coated with goat anti-mouse IgG (C line). When an adequate volume of test specimen is applied into the sample well of the strip, a burgundy colored C line should always be visible regardless of the color development on the T line. If the C line does not develop in a panel, the test result is invalid and the specimen must be retested with another device. An invalid result in one panel does not invalidate the test result in the other panel.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. 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 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 results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. 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. The positive and negative controls should be kept at 2°C-8°C or the temperature recommended**.** 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 bio-safety 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 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 on result interpretation.

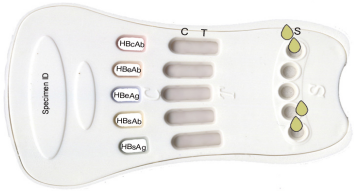
ASSAY PROCEDURE

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



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 the result window within 30 seconds, which could occur with a highly viscous specimen.

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

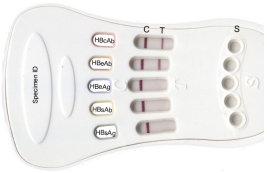
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen. If the C line does not develop, review the whole procedure and repeat test with a new device.

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°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

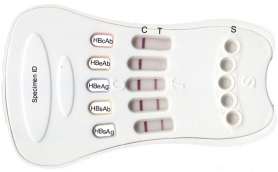
1. **NEGATIVE RESULT**: If only the C line is developed on the HBsAg, HBsAb, HBeAg strip, or both the C and the T lines are developed on either the HBeAb or the HBcAb strip, the test indicates a negative result on the parameter being tested.

Strips	C Line	T Line
HBcAb	Visible	Visible
HBeAb	Visible	Visible
HBeAg	Visible	No band
HBsAb	Visible	No band
HBsAg	Visible	No band



2. **POSITIVE RESULT**: If both the C and the T lines are developed on the HBsAg, HBsAb, or the HBeAg strip or only the C line is developed on the HBeAb or the HBcAb strip, the test indicates presence of the parameter being tested. The result is positive.

Strips	C Line	T Line
HBcAb	Visible	No band
HBeAb	Visible	No band
HBeAg	Visible	Visible
HBsAb	Visible	Visible
HBsAg	Visible	Visible



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 line is developed, the assay on the strip is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

Strips	C Line	T Line
HBcAb	No band	regardless
HBeAb	No band	regardless
HBeAg	No band	regardless
HBsAb	No band	regardless
HBsAg	No band	regardless

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

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 586 patient samples from susceptible subjects were tested by the *OnSite* HBV-5 Rapid Test and by other individual commercial rapid tests. The samples contained at least 120 positive samples for each test strip as well as HAV, HCV, HEV and RF positive samples to test for cross-reactivity. The relative sensitivity, relative specificity, and overall agreement for each is shown below.

Test	Relative Sensitivity	Relative Specificity	Overall Agreement
HBsAg	100%	100%	100%
HBsAb	100%	99.5%	99.7%
HBeAg	100%	100%	100%
HBeAb	100%	100%	100%
HBcAb	100%	99.6%	99.8%
Overall agreement: 99.5%			

2. **Analytical Sensitivity**

The detection limit for the HBsAg test is 1 ng/ml.
The detection limit for the HBsAb test is 30 mIU/ml.
The detection limit for the HBeAg test is 2 NCU/ml.
The detection limit for the HBeAb test is 2 NCU/ml.
The detection limit for the HBcAb test is 2 NCU/ml.

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, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* HBV-5 Rapid Test is limited to the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma. The intensities of the test lines do not have linear correlations with the antibody titers in the specimen.
3. A negative result for an individual subject indicates absence of detectable HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma. However, 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 the HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma present in the specimen is below the detection limits of the assays or the antibodies/antigens 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. 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
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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
30°C 2°C	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
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Tel: 858-457-8698
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PI-R0049C Rev. D
Effective date: 2013-06-28

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