Reliable Pro-detect Biomedicals Pvt. Ltd.
In vitro Diagnostic

INTENDED USE

The *Reliable one step* HBsAg Rapid Test device 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 *Reliable* one step HBsAg Rapid Test device 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].

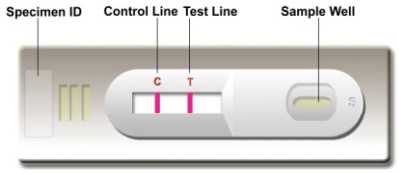
HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase[2], the core antigen (HBcAg) [3] and the e antigen (HBeAg) [4]. The core of HBV is enclosed in a coat that contains lipid, protein and carbohydrate and expresses an antigen terms hepatitis B surface antigen (HBsAg) [3].

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. 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 presists 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 *Reliable one step* HBsAg Rapid Test device detects HBsAg in serum or plasma in less than 15 minutes by untrained or minimally skilled personnel, without laboratory equipment.

TEST PRINCIPLE

The *Reliable one step* HBsAg Rapid Test device is a lateral flow chromatographic immunoassay. The test device consists of: 1) a burgundy colored conjugate containing mouse anti-HBsAg antibody conjugated with colloid gold (HBsAg Ab conjugates), 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with non-conjugated HBsAg antibody, and the C bands pre-coated with goat anti-mouse IgG antibody.



When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the test cassette. 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 burgundy colored T band, indicating a 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 / HBsAg Ab-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.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 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).

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 conduet the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15℃-30°C) before use.
5. Do not use the components in any other type of test kits 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 specimen or kit reagents are being handled.
10. Dispose of all specimens and materials used to perfrom the test as biohazardous 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. Read 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 device unopened at 2℃-30℃. The positive and negative controls should be kept at 2℃-8℃ . If stored at 2℃-8℃, 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℃.

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 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℃ to 8℃ if not tested immediately.

Store specimens at 2℃ to 8℃ up to 5 days. The spe cimens should be frozen at -20℃ for longer storage

Avoid multiple freeze-thaw cycles. Prior to te, 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.

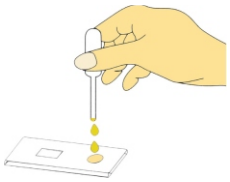
ASSAY PROCEDURE

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 plastic dropper with the specimen Holding the dropper vertically, dispense 5-8 drops (about 60- 90 µL) of specimen into the sample well 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 within 30 seconds in the result window, 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 results after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

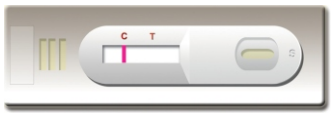
Using individual *Reliable one step* HBsAg Rapid Test device as described in the assay Procedure above, run 1 Positive Control and 1 Negative control (provided upon request) under the following circumstances to monitor test performance:

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℃ -30℃.
5. The temperature of the test area falls out side of 15℃ -30℃

Expected results are as follows:

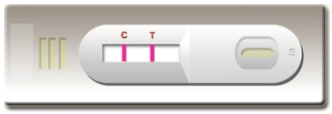
Negative Control

Only the C band shows color development. The T band shows no color development.



Positive Control

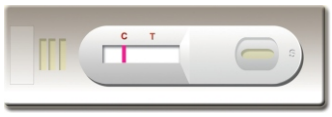
Both C and T bands show color development.



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 .5 ng/mL). The result is negative.



2. **POSITIVE RESULT** : If both C and T bands are developed, the test indicated that the specimen contains HBsAg at the level equal or higher than .5 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 *Reliable one step* HBsAg Rapid Test and by HBsAg ELISA kit with the test sensitivty at 0.5 ng/mL. Comparison for all subjects is showed in the following table.

	Reliable one step **HBsAg Rapid Test**		
HBsAg ELISA	**Positive**	**Negative**	**Total**
Positive	48	2	50
Negative	0	450	450
Total	48	452	500

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 *Reliable one step* HBsAg Rapid Test device is limited to the qualitative detection of HBsAg in human serum or plasma. The intensity of the test band dose 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 .5 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 be only 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, Purcell RH, Robinson WS. 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

Mfd. & Mktd. By :
Reliable Pro-detect Biomedicals Pvt. Ltd.
91C, Industrial Estate, Shoghi, Shimla (HP) India
Customer care No.: +91-1772860656