



INTENDED USE

Diagnocure 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 level equal to or higher than 1 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 *Diagnocure* HBsAg 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 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 polymerase², 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 *Diagnocure* 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 *Diagnocure* HBsAg Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy 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 strip 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 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 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 burgundy colored line of the immunocomplex of the control antibodies, regardless of color any 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 foil pouches containing:
 - a. One cassette device
 - b. One desiccant
2. Plastic droppers
3. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

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 may lead to 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 (15-30°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 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 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the result after 20 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 4-30°C. If stored at 4-8°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 over 30°C.

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 venipuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into a 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.

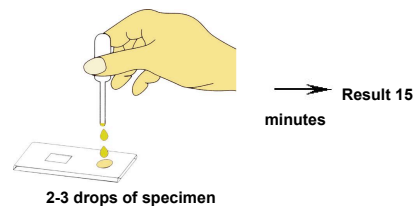
Test specimens as soon as possible after collecting. Store specimens at 2-8°C if not tested immediately. Specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

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: 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 the specimen ID number.
- Step 4: Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 2-3 drops (about 50-70 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 clinics not provided in the kit) to 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: Result can be read in 15 minutes. Positive results may be visible in as soon as 1 minute.

Do not read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

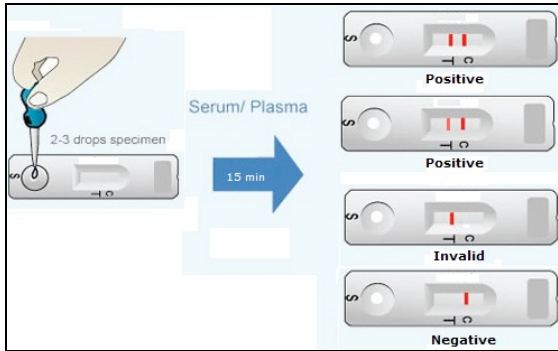
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding 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 ensure the proper performance of the assay, particularly under the following circumstances:

- a. A new operator uses the kit prior to performing the 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, 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 are developed, 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.
Samples with reactive 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 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 samples from susceptible subjects were tested with the *Diagnocure* 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.

HBsAg ELISA	Diagnocure HBsAg Rapid Test		Total
	Positive	Negative	
Positive	97	0	97
Negative	0	463	463
Total	97	463	560

Relative Sensitivity: 100% , Relative Specificity: 100%, Overall Agreement: 100%

2. Cross-Reactivity

Cross-reactivity with specimens from other infectious diseases:

Specimen	Sample Size	HBsAg Reactivity
Dengue Positive Serum	10	Negative
HAV Positive Serum	10	Negative
HCV Positive Serum	10	Negative
HIV Positive Serum	10	Negative
Syphilis Positive Serum	10	Negative
TB Positive Serum	10	Negative
H. pylori Positive Serum	10	Negative
ANA Positive Serum	6	Negative
HAMA Positive Serum	4	Negative
RF Positive Serum (≤2,500 IU/ml)	3	Negative

3. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the *Diagnocure* 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 *Diagnocure* HBsAg Rapid Test.

Potential Interfering Substances Spiked	HBsAg Reactivity		
	Negative	Weak Positive	Medium Positive
Control	-	+	++
Bilirubin 20 mg/dL	-	+	++
Creatinine 442 µmol/L	-	+	++
Glucose 55 mmol/L	-	+	++
Albumin 50 g/L	-	+	++
Salicylic Acid 4.34 mmol/L	-	+	++
Heparin 3,000 U/L	-	+	++
EDTA 3.4 µmol/L	-	+	++
Human IgG 1,000mg/dL	-	+	++
Sodium citrate 3.8%	-	+	++

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 *Diagnocure* 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. If the symptoms persist when the result from *Diagnocure* 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, 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. Magnuson LO, Espmark A. A new antigen complex co-occurring with Australia antigen. Acta Pathol Microbiol Scand [B] Microbiol Immunol. 1972;80(2):335-7.

Note: - : Negative; + : Positive; ++ : Medium Positive