



One Step HBV-5 Combo Rapid Test

(Whole Blood/Serum/Plasma)

A rapid test for the qualitative detection of hepatitis B virus markers, Hepatitis B Surface Antigen (HBsAg), Hepatitis B Surface Antibody (HBsAb), Hepatitis B Envelope Antigen (HBeAg), Hepatitis B Envelope Antibody (HBeAb), and Hepatitis Core Antibody (HBcAb) in human whole blood, serum, or plasma specimens.

For professional *in vitro* diagnostic use only.

Please read the package insert carefully before using.

【SPECIFICATION】

10 Tests/Kit

【INTENDED USE】

The iCARE One Step HBV-5 Combo Rapid Test is a serological, lateral flow chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg), Hepatitis B Surface Antibody (HBsAb), Hepatitis B Envelope Antigen (HBeAg), Hepatitis B Envelope Antibody (HBeAb), and Hepatitis Core Antibody (HBcAb) in human whole blood, serum, or plasma specimens to aid in the diagnosis of infection with hepatitis B virus (HBV). The test only provides preliminary analysis results but not critical diagnosis criteria. Any reactive specimen with the iCARE One Step HBV-5 Combo Rapid Test must be analyzed and confirmed with alternative testing method(s) and clinical findings. The test is intended for healthcare professional use.

【SUMMARY】

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). HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase, the core antigen (HBcAg) and the e antigen (HBeAg). The core of HBV is enclosed in a coat that contains lipid, carbohydrate and protein including an antigen termed hepatitis B surface antigen (HBsAg). Most people fight off the infection themselves, but approximately 5-10 percent of those infected with the virus become carriers, and an additional 5-10 percent of those infected each year will progress to chronic liver disease, cirrhosis and possibly liver cancer.

The iCARE One Step HBV-5 Combo Rapid Test utilizes specific antigens and antibodies to detect the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb qualitatively in human whole blood, serum, or plasma specimens. This assay can be performed to get test result at 15-20 minutes by minimally trained personnel and without cumbersome laboratory equipment.

【TEST PRINCIPLE】

The iCARE One Step HBV-5 Combo Rapid Test is a qualitative membrane-based immunoassay for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human whole blood, serum, or plasma specimens.

HBsAg and HBeAg

The HBsAg and HBeAg tests are qualitative, two-site sandwich immunoassays for the detection of HBsAg or HBeAg in whole blood, serum or plasma specimens. The membrane is pre-coated with anti-HBsAg or anti-HBeAg antibodies on the test line region (T) of the strip. During testing, the specimen reacts with the particle coated with anti-HBsAg or anti-HBeAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg or anti-HBeAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test line region (T) indicates a positive result, while its absence indicates a negative result.

HBsAb

Hepatitis B surface Antibody (HBsAb) is also known as antiHepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in whole blood, serum or plasma specimens. The membrane is pre-coated with HBsAg on the test line region (T) of the strip. During testing, the specimen reacts with the particle coated with HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane and generate a colored line. The presence of this colored line in the test line region (T) indicates a positive result, while its absence indicates a negative result.

HBeAb and HBcAb

Hepatitis B envelope Antibody (HBeAb) is also known as antiHepatitis B envelope Antigen (anti-HBe). Hepatitis B core Antibody (HBcAb) is also known as anti-Hepatitis B core Antigen (anti-HBc). These tests are immunoassays based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. The membrane is pre-coated with HBeAg or HBcAg on the test line region (T) of the strip. During testing, anti-HBe antibody or anti-HBc antibody, if present in the specimen, will compete with particle coated anti-HBe antibody or anti-HBc antibody for limited amount of HBeAg or all HBcAg on the membrane, and no line will form in the test line region (T), indicating a positive result. A visible colored line will form in the control line (C) and test line region (T) if there is no anti-HBe antibody or anti-HBc antibody in the specimen because all the antibody coated particles will be captured by the antigen coated in the test line region (T).

An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control lines, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.

【MATERIALS PROVIDED】

- Test device individually foil pouched with a desiccant
- Dropper (30 uL)
- Sample diluent
- Package insert

【MATERIALS REQUIRED BUT NOT PROVIDED】

Timer, Positive control, Negative control, Specimen collection containers

【WARNINGS AND PRECAUTIONS】

1. For *in vitro* diagnostic use only. Do not reuse the test.
2. Do not freeze the test kit or its components.
3. These instructions must be carefully read and strictly followed by a trained healthcare professional to achieve accurate results. All users should read the instructions before performing test.
4. The test is only for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, not for any other viruses or pathogens.
5. Inadequate or inappropriate specimen collection, storage, and transportation are likely to result in false negative test results.
6. Do not use hemolyzed blood specimens for testing.
7. Do not eat, drink or smoke in the area where handling specimens or performing the test.
8. Do not use the test kit beyond its expiration date.
9. Do not mix components from different kit lots.
10. Leave test device sealed in its foil pouch until just before use. Do not use the test device if the pouch is damaged or the seal is broken.

- To avoid contamination or inaccurate test result, do not touch the reaction area of test device when performing the test.
- Wear appropriate personal protection equipment and gloves when performing the test, collecting and handling patient specimens.
- Dispose of all used test devices and potentially contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local laws and regulations.

【STORAGE AND STABILITY】

- The test kit should be stored either at room temperature or refrigerated (2-30°C), away from direct sunlight. Do not freeze the kit or expose the kit to temperatures over 30°C.
- The shelf life of the kit is as indicated on the outer package (24 months from date of manufacture).
- This test kit is stable until the expiration date marked on the outer package and foil pouch. Ensure all test components are at room temperature (15-30°C) before use.
- Perform the test immediately after taking out the test device from the foil pouch.

【SAMPLE COLLECTION AND PREPARATION】

Consider any materials of human origin as infectious and handle them using standard biosafety procedures. The test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma specimens. Follow standard laboratory procedures to collect specimens.

Plasma/Serum

- Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
- To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
- 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. Specimens can be stored at 2-8°C for up to 3 days, and should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles (no more than 3 times). Prior to testing, equilibrate 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 so as to avoid interference on result interpretation.

Whole Blood

Collect whole blood by either fingertip puncture or by venipuncture into collection tube containing EDTA, citrate or heparin for plasma. Do not use any hemolyzed blood for testing.

Do not freeze a whole blood specimen, otherwise the red blood cell will break, which may cause hemolysis. Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours after collection.

【TEST PREPARATION】

Before testing, open the package and equilibrate the test device, sample diluent, specimens and/or controls to room temperature, and shake the sample diluent gently before use. The most suitable temperature condition to perform the test is room temperature (15-30°C). If the test kit is stored at room temperature, it can be opened and used immediately.

【TEST PROCEDURES】

- Take out the test device from sealed foil pouch and place on a dry, clean, and level surface.
- Be sure to label the device with specimen's ID number.
- Fill the pipette dropper with the specimen. Hold the dropper vertically and transfer one drop of whole blood/serum/plasma specimen (approximately 30 µL) into each specimen well (S) making sure that there are no air bubbles. Then add two drop of sample diluent (approximately 80-100 µL) to each specimen well (S) immediately. See illustration below.
- Start the timer.
- Wait for the colored line(s) to appear. Read test results at 15 minutes. Do not interpret the result after 20 minutes.



【INTERPRETATION OF TEST RESULTS】

(Please refer to the illustrations below)

Warning: Do not interpret all 5 test strips at the same criterion. Please follow the directions below carefully.

For HBsAg, HBsAb, HBeAg

POSITIVE:* Two distinct colored lines appear. One colored line should be in the control region (C) and another colored line should be in the test region (T).

***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of HBsAg, HBsAb, HBeAg present in the specimen. Therefore, any shade of color in the test region (T) should be considered positive.

NEGATIVE: One colored line appears in the control region (C). No apparent colored line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

For HBeAb, HBcAb

NEGATIVE:* Two distinct colored lines appear. One colored line should be in the control region (C) and another colored line should be in the test region (T).

***NOTE:** The intensity of the color in the test line region (T) may vary. But it should be considered negative whenever there is even a faint pink line.

POSITIVE: One colored line appears in the control region (C). No apparent colored line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



【QUALITY CONTROL】

- Internal Control:** An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control line, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.
- External Control:** Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

- The test is only used for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human whole blood, serum, or plasma specimens by healthcare professionals. The intensity of the test line does not have a linear correlation with the HBsAg, HBsAb, HBeAg, HBeAb and HBcAb level in the specimen.
- The test does not indicate the level of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in the specimens or the rate of increase in HBsAg, and should not be used as the sole criteria for the diagnosis of infection with HBV.
- A negative result indicates that HBsAg, HBsAb, HBeAg, HBeAb and HBcAb is not present in the specimen. However, a negative test result at any time does not preclude the possibility of exposure to or infection with HBV.
- A negative result may occur if the level of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb present in the specimen is below the detection limits of the assay or the antigens/antibodies that are detected are not present during the stage of disease when a sample is collected.
- A positive result using the iCARE One Step HBV-5 Combo Rapid Test suggests the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in the sample and the positive test result should be interpreted as preliminary positive for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. Positive test results must be confirmed by additional testing.
- If the test result is negative and clinical symptoms persist, re-sample the patient and additional testing using alternative clinical methods is recommended.
- Test results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

【PERFORMANCE CHARACTERISTIC】

1. Clinical Performance

The iCARE One Step HBV-5 Combo Rapid Test has correctly identified specimens of a performance panel and has been evaluated with a reference commercial assay using clinical specimens, respectively. Test results are presented in the table below.

Clinical performance compared to ELISA: HBsAg

iCARE One Step HBV-5 Combo Rapid Test	ELISA		
	Positive	Negative	Total
Positive	710	5	715
Negative	10	2120	2130
Total	720	2125	2845

Sensitivity (Positive Percent Agreement): 98.6% = 710/720 (95% CI: 97.46%~99.24%)
 Specificity (Negative Percent Agreement): 99.7% = 2120/2125 (95% CI: 99.45%~99.90%)
 Accuracy (Overall Percent Agreement): 99.4% = (710+2120)/2845 (95% CI: 99.13%~99.68%)

Clinical performance compared to ELISA: HBsAb

iCARE One Step HBV-5 Combo Rapid Test	ELISA		
	Positive	Negative	Total
Positive	678	2	680
Negative	2	708	710
Total	680	710	1390

Sensitivity (Positive Percent Agreement): 99.7% = 678/680 (95% CI: 98.93%~99.92%)
 Specificity (Negative Percent Agreement): 99.7% = 708/710 (95% CI: 98.98%~99.92%)
 Accuracy (Overall Percent Agreement): 99.7% = (678+708)/1390 (95% CI: 99.26%~99.89%)

Clinical performance compared to ELISA: HBeAg

iCARE One Step HBV-5 Combo Rapid Test	ELISA		
	Positive	Negative	Total
Positive	610	8	618
Negative	12	900	912
Total	622	908	1530

Sensitivity (Positive Percent Agreement): 98.0% = 610/622 (95% CI: 96.66%~98.99%)
 Specificity (Negative Percent Agreement): 99.1% = 900/908 (95% CI: 98.27%~99.55%)
 Accuracy (Overall Percent Agreement): 98.6% = (610+900)/1530 (95% CI: 97.99%~99.15%)

Clinical performance compared to ELISA: HBeAb

iCARE One Step HBV-5 Combo Rapid Test	ELISA		
	Positive	Negative	Total
Positive	489	7	496
Negative	11	793	804
Total	500	800	1300

Sensitivity (Positive Percent Agreement): 97.8% = 489/500 (95% CI: 96.66%~98.99%)
 Specificity (Negative Percent Agreement): 99.1% = 793/800 (95% CI: 98.27%~99.55%)
 Accuracy (Overall Percent Agreement): 98.6% = (489+793)/1300 (95% CI: 97.99%~99.15%)

Clinical performance compared to ELISA: HBcAb

iCARE One Step HBV-5 Combo Rapid Test	ELISA		
	Positive	Negative	Total
Positive	547	8	555
Negative	13	812	825
Total	560	820	1380

Sensitivity (Positive Percent Agreement): 97.6% = 547/560 (95% CI: 96.07%~98.64%)
 Specificity (Negative Percent Agreement): 99.0% = 812/820 (95% CI: 98.09%~99.50%)
 Accuracy (Overall Percent Agreement): 98.4% = (547+812)/1380 (95% CI: 97.68%~99.00%)

2. Analytical Sensitivity

The analytical sensitivity of the iCARE One Step HBV-5 Combo Rapid Test was evaluated by testing reference panel. The results demonstrated that the assay could detect a concentration of 0.5ng/mL (1.0 IU/mL) HBsAg, 10 mIU/mL HBsAb, 2.0 NCU/mL HBeAg, 2.0 NCU/mL HBeAb, 2.0 NCU/mL HBcAb.

3. Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of three specimens containing negative, low positive, high positive of HBsAg, HBsAb, HBeAg, HBeAb, HBcAb. The negative and positive values were correctly identified 99% of the time.

Inter-Assay

Between-run precision has been determined by using the same three specimens of negative, low positive, high positive of HBsAg, HBsAb, HBeAg, HBeAb, HBcAb in 15 independent assays. Three different lots of the iCARE One Step HBV-5 Combo Rapid Test has been tested over a 10 days period using negative, low positive and high positive specimens. The specimens were correctly identified 99% of the time.

4. Cross-reactivity

The iCARE One Step HBV-5 Combo Rapid Test has been tested by HAMA, Rheumatoid factor (RF), HAV, Syphilis, HIV, H. Pylori, HCV, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

5. Interfering Substances

The iCARE One Step HBV-5 Combo Rapid Test has been tested for possible interference from visibly hemolyzed and lipemic specimens. No interference was observed. In addition, no interference was observed in specimens containing up to 2,000 mg/dL Hemoglobin, 1,000 mg/dL Bilirubin, and 2,000 mg/dL human serum Albumin.

【REFERENCES】

- 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.
- 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.
- Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet. 1970; 1(7649):695-8.
- 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.
- Kao JH. Diagnosis of hepatitis B virus infection through serological and virological markers. Expert Rev Gastroenterol Hepatol. 2008;2(4):553-562.

【INDEX OF SYMBOLS】

	Consult instruction for use		For <i>in vitro</i> diagnostic use only		Catalog number		Temperature limit
	Lot number		Use by		Do not reuse		Contains sufficient for <X> tests
	Keep dry		Manufacturer		Date of manufacture		Keep away from sunlight
	Do not use if package is damaged						

