

A rapid test 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 serum or plasma.

For professional *in vitro* diagnostic use only.

INTENDED USE

The HBV Combo Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in serum or plasma.

SUMMARY

Chronic hepatitis B is a serious, debilitating illness that can cause cirrhosis of the liver, liver cancer and death. Chronic hepatitis B is the main cause of liver cancer and the tenth leading cause of death worldwide, with 400,000,000 people infected with the virus. Every year, one million people worldwide are expected to die from this infection. 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 HBV Combo Rapid Test (Serum/Plasma) is a rapid test to qualitatively detect the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in serum or plasma without the use of an instrument.¹

PRINCIPLES

HBsAg and HBeAg

The HBsAg and HBeAg tests are qualitative, two-site sandwich immunoassays for the detection of HBsAg or HBeAg in serum or plasma. The membrane is pre-coated with anti-HBsAg or anti-HBeAg antibodies on the test line region of the strip. During testing, the serum or plasma 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 indicates a positive result, while its absence indicates a negative result.

HBsAb

Hepatitis B surface Antibody (HBsAb) is also known as anti-Hepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in serum or plasma. The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the serum or plasma 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 indicates a positive result, while its absence indicates a negative result.

HBeAb and HBcAb

Hepatitis B envelope Antibody (HBeAb) is also known as anti-Hepatitis 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 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 HBcAg on the membrane, and no line will form in the test line region, indicating a positive result. A visible colored line will form in the test line region 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.

To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

Materials provided

- Test cassettes
- Droppers
- Package insert
- Specimen collection containers
- Centrifuge
- Timer

Materials required but not provided

WARNINGS AND PRECAUTIONS

Please read all the information in this package insert before performing the test.

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

Store as packaged at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

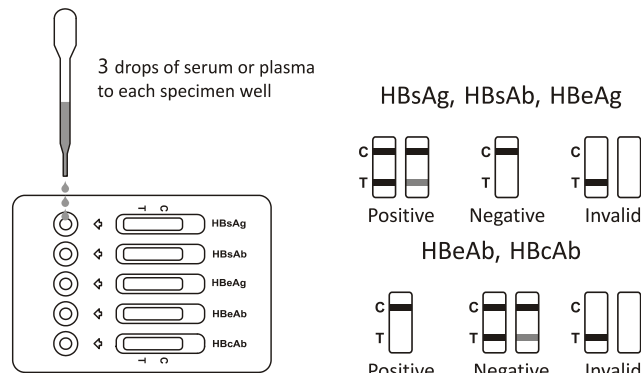
SPECIMEN COLLECTION AND PREPARATION

- The HBV Combo Rapid Test can be performed using either serum or plasma.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

DIRECTIONS FOR USE

- Remove the test cassette from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
- Place the test cassette on clean and level surface. Hold the dropper vertically and transfer **3 full drops of serum or plasma** (approx.75 µL) to each sample well of the test cassette respectively, then start the timer. Avoid trapping air bubbles in the specimen well. See the illustration below.

- Wait for the red line to appear. **The result should be read at 15 minutes.** Do not interpret the results after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

Warning: Do not interpret all 5 tests with the same criterion. Carefully follow the directions below.

HBsAg, HBsAb, HBeAg

POSITIVE: * **Two 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 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.

HBeAb, HBcAb

NEGATIVE: * **Two 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 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

A procedural control is included in the test. A red line appearing in the control line region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

- The HBV Combo Rapid Test is for professional *in vitro* diagnostic use only. The test should be used for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in serum or plasma specimen. Neither the quantitative value nor the rate of HBsAg, HBsAb, HBeAg, HBeAb, HBcAb concentration can be determined by this qualitative test.
- The HBV Combo Rapid Test will only indicate the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis B viral infection.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HBV Combo Rapid Test (Serum/Plasma) was compared with leading commercial EIA/RIA HBsAg, HBsAb, HBeAg, HBeAb, HBcAb tests, the results show that the HBV Combo Rapid Test (Serum/Plasma) has a high sensitivity and specificity.

HBsAg

Method	EIA		Total Results
	Results	Positive	Negative
HBsAg Rapid Test (Serum/Plasma)	Positive	241	2
	Negative	0	359
Total Results		241	361
		602	

Relative Sensitivity: >99.9% (95%CI*: 98.8%-100%)

Relative Specificity: 99.4% (95%CI*: 98.0%-100%)

Accuracy: 99.7% (95%CI*: 98.8%-100%)

*Confidence Intervals

HBsAb

Method	RIA		Total Results
	Results	Positive	Negative
HBsAb Rapid Test (Serum/Plasma)	Positive	194	9
	Negative	7	391
Total Results		201	400
		601	

Relative Sensitivity: 96.5% (95%CI*: 93.0%-98.6%)

Relative Specificity: 97.8% (95%CI*: 95.8%-99.0%)

Accuracy: 97.3% (95%CI*: 95.7%-98.5%)

*Confidence Intervals

HBeAg

Method	RIA		Total Results
	Results	Positive	Negative
HBeAg Rapid Test (Serum/Plasma)	Positive	154	9
	Negative	6	429
Total Results		160	438
		598	

Relative Sensitivity: 96.3% (95%CI*: 92.1%-98.6%)

Relative Specificity: 97.9% (95%CI*: 96.1%-99.1%)

Accuracy: 97.5% (95%CI*: 95.9%-98.6%)

*Confidence Intervals

HBeAb				
Method		EIA		Total Results
HBeAb Rapid Test (Serum/Plasma)	Results	Positive	Negative	
	Positive	146	7	153
	Negative	4	329	333
Total Results		150	336	486

Relative Sensitivity: 97.3% (95%CI*: 93.3%-99.3%)

Relative Specificity: 97.9% (95%CI*: 95.8%-99.2%)

Accuracy: 97.7% (95%CI*: 96.0%-98.9%)

*Confidence Intervals

HBcAb				
Method		EIA		Total Results
HBcAb Rapid Test (Serum/Plasma)	Results	Positive	Negative	
	Positive	358	4	362
	Negative	8	167	175
Total Results		366	171	537

Relative Sensitivity: 97.8% (95%CI*: 95.7%-99.1%)

Relative Specificity: 97.7% (95%CI*: 94.1%-99.4%)

Accuracy: 97.8% (95%CI*: 96.1%-98.8%)

*Confidence Intervals

Limitation of Detection

The minimum detection level is 1ng/ml for HBsAg positive specimen, 10mIU/mL for HBsAb positive specimen, 5IU/ml for HBeAg positive specimen, 6IU/ml for HBeAb positive specimen and 5mIU/ml for HBcAb positive specimen.

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 in15 independent assays. Three different lots of the HBV Combo Rapid Test (Serum/Plasma) 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.

Cross-reactivity

The HBV Combo Rapid Test (Serum/Plasma) has been tested by HAMA, Rheumatoid factor (RF), HAV, Syphilis, HIV, *H.Pylori*, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity

Interfering Substances

The HBV Combo Rapid Test (Serum/Plasma) 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.

BIBLIOGRAPHY

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INDEX OF SYMBOLS			
	Consult instructions for use		Contains sufficient for <n> tests
	In vitro diagnostic medical device		Use-by date
	Temperature limit		Batch code
	Do not use if package is damaged		Manufacturer
	Catalogue number		Do not re-use