HBV COMBO DEVICE (2-30°C)

CATALOGUE NUMBER	KIT SIZE (TESTS)
RADHBV1	20 Tests

Intended Use:
The HBV One Step Hepatitis B Virus Combo Test Device (Serum/Plasma) 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 One Step Hepatitis B Virus Combo Test Device (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.

Test Principle:
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.2 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 coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result.
HBsAb
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 coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result.
HBeAb and HBcAb
These tests are immunoassays based on the principle of competitive binding. During testing, the sample is combined with with particle coated anti-HBe antibody and anti-HBc antibody on the membrane at the sample well. 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. If present in the sample, anti-HBe antibody or anti-HBc antibody will compete with the labelled particle anti-HBe antibody or anti-HBc antibody for limited amount of HBeAg or HBcAg at the test region membrane, and no line will form in the test line region, indicating a positive result. A visible coloured 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 labelled particles will be captured by the antigen.
To serve as a procedural control, a coloured line should always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Materials Provided
Individually pouched test devices
Disposable pipettes
Instructions for Use sheet

Materials not provided: Timer, specimen collection container, centrifuge

Precautions:
- For professional *in vitro* diagnostic use only.
- Do not use after the expiry date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded according to local regulations.

Reagent Preparation and Stability:

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

Specimen Collection and Storage:
- The HBV One Step Hepatitis B Combo Test Device (Serum/Plasma) can be performed using either serum or plasma.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non- hemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. 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 local regulations for the transportation of etiologic agents.

Assay Procedure:

Bring tests, specimens, and/or controls to room temperature (15-30°C) before use.
1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed within one hour.
2. Using the provided disposable pipette, transfer 3 drops of serum or plasma (approximately 75 µL) to each specimen well (S) of the device respectively, then start the timer.
   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result window.
   As the test begins to work, colour will migrate across the membrane.
3. Wait for the coloured band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

Interpretation of Results:

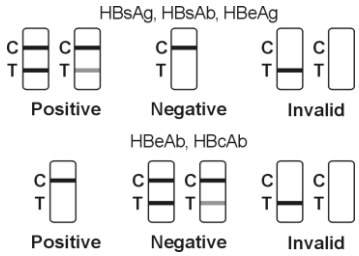
HBsAg, HBsAb, HBeAg
POSITIVE: *Two coloured lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).
NEGATIVE: One coloured line appears in the control line region (C). No evidence of a coloured line in the test line region (T).
***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentrations of HBsAg, HBsAb, and HBeAg present in the specimen. Therefore, any shade of colour in the test line region (T) should be considered positive.
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. the Review procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

HBeAb, HBcAb

POSITIVE: One coloured line appears in the control line region (C), No evidence of a coloured line in the test region (T).

NEGATIVE: *Two coloured lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).
***NOTE:** The shade of colour in the test line region (T) may vary, but it should be considered negative whenever there is even a faint development of a line.

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 device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



Quality Controls:

- A procedural control is included in the test. A coloured 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 of the Test:
1. The HBV One Step Hepatitis B Virus Combo Test Device (Serum/Plasma) is for in vitro diagnostic use only. This test should be used for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in serum or plasma. Neither the quantitative value nor the rate of increase in the concentration of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb can be determined by this qualitative test.
2. The HBV One Step Hepatitis B Virus Combo Test Device (Serum/Plasma) 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.
3. Diagnosis should not be made on the results of this test alone but be considered along with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Hepatitis B Virus infection.

Performance Characteristics:
Sensitivity and Specificity
The HBV One Step Hepatitis B Combo Test Device (Serum/Plasma) was compared with leading commercial EIA/RIA HBsAg, HBsAb, HBeAg, HBeAb, HBcAb tests, the results show that the HBV One Step Hepatitis B Combo Test Device (Serum/Plasma) has a high sensitivity and specificity.

HBsAg

Method		HBsAg ELISA		Total Results
HBV Combo Rapid Test Device	Result	Positive	Negative	
	Positive	241	2	243
	Negative	0	359	359
Total Results		241	361	602

Relative Sensitivity: >99.9% (95%CI*: 98.8% - 100%) *Confidence Interval
Relative Specificity: 99.4% (95%CI*: 98.0% - 100%)
Accuracy: 99.7% (95%CI*: 98.8% - 100%)

HBsAb

Method		HBsAg ELISA		Total Results
HBV Combo Rapid Test Device	Result	Positive	Negative	
	Positive	194	9	203
	Negative	7	391	398
Total Results		201	400	601

Relative Sensitivity: 96.5% (95%CI*: 93.0% - 98.6%) *Confidence Interval
Relative Specificity: 97.8% (95%CI*: 95.8% - 99.0%)
Accuracy: 97.3% (95%CI*: 95.7% - 98.5%)

HBeAg

Method		HBsAg ELISA		Total Results
HBV Combo Rapid Test Device	Result	Positive	Negative	
	Positive	154	9	163
	Negative	6	429	435
Total Results		160	438	598

Relative Sensitivity: 96.3% (95%CI*: 92.1% - 98.6%) *Confidence Interval
Relative Specificity: 97.9% (95%CI*: 96.1% - 99.1%)
Accuracy: 97.5% (95%CI*: 95.9% - 98.6%)

HBeAb

Method		HBsAg ELISA		Total Results
HBV Combo Rapid Test Device	Result	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%) *Confidence Interval
Relative Specificity: 97.9% (95%CI*: 95.8% - 99.2%)
Accuracy: 97.7% (95%CI*: 96.0% - 98.9%)

HBcAb

Method		HBsAg ELISA		Total Results
HBV Combo Rapid Test Device	Result	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%) *Confidence Interval
Relative Specificity: 97.7% (95%CI*: 94.1% - 99.4%)
Accuracy: 97.8% (95%CI*: 96.1% - 98.8%)

Precision

Intra-Assay

Within-run precision was determined using 15 replicates of three samples containing negative, low positive and high positive concentrations of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. The negative and positive results were correctly identified 99 % of the time.

Inter-Assay

Between-run precision was determined by running 15 separate assays on 15 different days on three samples containing negative, low positive and high positive concentrations of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. Three different lots of the HBV One Step Hepatitis B Virus Combo Test Device were tested using these samples. The specimens were correctly identified 99 % of the time.

Cross-Reactivity

The HBV One Step Hepatitis B Virus Combo Test Device has been tested using HAV, HIV, RF, Syphilis, HAM, MONO, CMV, Rubella, Toxo and *H. pylori* positive samples and the results showed no cross-reactivity.

Interfering Substances

The HBV One Step Hepatitis B Virus Combo Test Device was tested for interference by visibly haemolysed and lipemic samples. No interference was observed. There was no interference from samples containing up to 2000 mg/dl haemoglobin, 1000 mg/dl bilirubin and 2000 mg/dl albumin.

References:

1. 1. Chizzali-Bonfadin C., Addlassnig K.P., Kreihsl M., Hatvan A., Horak W., Knowledge-based interpretation of serologic tests for hepatitis on *the World Wide Web.* Clin Perform Qual Health Care 1997 Apr-Jun 5:61-3

2. ter Bog F., ten Kate F.J., Cuypers H.T., Leentvaar-KuipersA., Oosting J., Wertheim-van Dillen P.M., Honkoop P, Rasch M.C., de Man R.A., vab Hattum J., Chamelueau R.A., Reesink H.W., Jones E.A., Relation between laboratory results and histological hepatitis activity in individuals postitve for hepatitis B surface antigen and antibodies to hepatitis B e antigen, Lancet 1998 June 351:1914-8

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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