

**OneStep  
 HBV Combo RapiCard™  
 InstaTest (Serum/Plasma)**

**REF 177471-1-44**

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 use only.

|                    |                      |
|--------------------|----------------------|
| <b>Sensitivity</b> | <b>96.3% ~ 99.9%</b> |
|--------------------|----------------------|

**IVD**  See external Label  2-30°C  Σ=1 Test

**INTENDED USE**

Cortez Diagnostics, Inc. OneStep HBV Combo RapiCard™ InstaTest (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 Combo RapiCard™ InstaTest (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.<sup>1</sup>

**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.<sup>2</sup>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 allHBcAg 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.

**REAGENTS**

The test cassette contains anti-HBsAg particles, HBsAg particles, anti-HBeAg particles, HBeAg particles respectively and anti-HBsAg, HBsAg, anti-HBeAg, HBeAg coated on the membrane respectively.

**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 RapiCard™ InstaTest (Serum/Plasma) 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.

**MATERIALS**

**Materials provided:**

- Test cassettes
- Droppers
- Package insert

**Materials required but not provided:**

- Specimen collection containers Centrifuge
- Timer

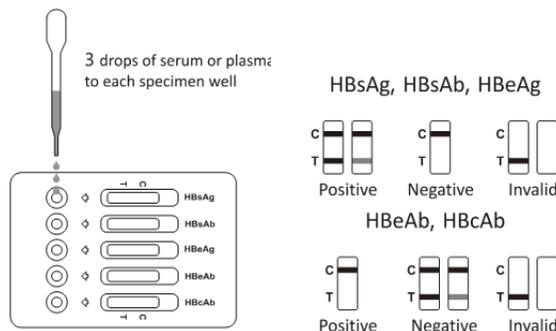
**PRECAUTION**

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

1. For professional in vitro diagnostic use only. Do not use after the expiration date.
2. Do not eat, drink or smoke in the area where the specimens or kits are handled.
3. Hand 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.
4. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
5. Humidity and temperature can adversely affect results.

**ASSAY PROCEDURE**

1. 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.
2. Place the test cassette on clean and level surface. Hold the dropper vertically and transfer 3 full drops of serum or plasma (approx. 75ul) 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.
3. Wait for the red line to appear. The result should be read at 15 minutes. Do not interpret the results after 20 minutes.



**RESULTS**

(Please refer to the illustration above)

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

**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**

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.

**LIMITATION**

1. The HBV Combo RapiCard™ InstaTest (Serum/ Plasma) 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.
2. The HBV Combo RapiCard™ InstaTest (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. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

## PERFORMANCE CHARACTERISTICS

### Sensitivity and Specificity

HBV Combo RapiCard™ InstaTest (Serum/ Plasma) was compared with leading commercial EIA/RIA HBsAg, HBsAb, HBeAg, HBeAb, HBcAbtests, the results show that the HBV Combo RapiCard™ InstaTest (Serum/ Plasma) has a high sensitivity and specificity.

#### HBsAg

| Method                                  | Results  | EIA      |          | Total Results |
|---|----------|----------|----------|---------------|
|   |          | Positive | Negative |               |
| HBsAg RapiCard™ InstaTest(Serum/Plasma) | Positive | 241      | 2        | 243           |
|   | Negative | 0        | 359      | 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                                  | Results  | RIA      |          | Total Results |
|---|----------|----------|----------|---------------|
|   |          | Positive | Negative |               |
| HBsAb RapiCard™ InstaTest(Serum/Plasma) | Positive | 194      | 9        | 203           |
|   | Negative | 7        | 391      | 398           |
| 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: 99.7% (95%CI\*: 98.8%-100%) \*Confidence Intervals

#### HBeAg

| Method                                  | Results  | RIA      |          | Total Results |
|---|----------|----------|----------|---------------|
|   |          | Positive | Negative |               |
| HBeAg RapiCard™ InstaTest(Serum/Plasma) | Positive | 154      | 9        | 163           |

|               | Negative | 6   | 429 | 435 |
|---------------|----------|-----|-----|-----|
| 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                                  | Results  | EIA      |          | Total Results |
|---|----------|----------|----------|---------------|
|   |          | Positive | Negative |               |
| HBeAb RapiCard™ InstaTest(Serum/Plasma) | 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                                  | Results  | EIA      |          | Total Results |
|---|----------|----------|----------|---------------|
|   |          | Positive | Negative |               |
| HBcAb RapiCard™ InstaTest(Serum/Plasma) | 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

### 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 HBV Combo RapiCard™

InstaTest (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 RapiCard™ InstaTest (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 RapiCard™ InstaTest (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, 1000 mg/dL Bilirubin, and 2000 mg/dL human serum Albumin.

## REFERENCES

- Chizzali-Bonfadin C., Adlassnig K.P., Kreihsl M., Hatvan A., Horak W., Knowledge-based interpretation of serologic tests for hepatitis on the World Wide Web. Clin Perform Qual HealthCare 1997 Apr-Jun 5:61-3
- ter Bog F., ten Kate F.J., Cuyper H.T., Leentvaar-Kuipers A., Oosting J., Wertheim-van Dillen P.M., Honkoop P, Rasch M.C., de Man R.A., van Hattum J., Chamelueau R.A., Reesink H.W., Jones E.A., Relation between laboratory results and histological hepatitis activity in individuals positive for hepatitis B surface antigen and antibodies to hepatitis B e antigen, Lancet 1998 June 351:1914-8

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| <p><b>ISO 13485</b><br/><b>ISO 9001</b></p>  <p> <b>Diagnostic Automation/<br/>Cortez Diagnostics, Inc.</b><br/> <b>21250 Califa St, Suite 102 and 116,<br/>Woodland Hills, California 91367 USA</b></p> |  |
| <b>Date Adopted</b>  | <b>2017-07-18</b>  |
| <b>REF</b> 177471-1-44   | <b>CORTEZ- OneStep<br/>HBV Panel (5 in 1 )<br/>RapiCard™ InstaTest</b> |
| Revision Date: <b>2017-04-13</b>   |  |