Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

OneStep HBsAg RapiCard™ InstaTest (Serum/Plasma/Whole Blood)

REF 176571-1-44

A rapid test for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in whole blood, serum or plasma.
For professional in vitro diagnostic use only.

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

INTENDED USE

The HBsAg RapiCard™ is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in whole blood,serum or plasma.

SUMMARY AND EXPLANATION

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis are caused by Hepatitis A virus, Hepatitis B virus (HBV) or Hepatitis C virus. The complex antigen found on the surface of HBV is called HBsAg. Previous designations included the Australia or Au antigen. The presence of HBsAg in whole blood,serum or plasma is an indication of an active Hepatitis B infection, either acute or chronic. In a typical Hepatitis B infection, HBsAg will be detected 2 to 4 weeks before the ALT level becomes abnormal and 3 to 5 weeks before symptoms or jaundice develop. HBsAg has four principal subtypes: adw, ayw, adr and ayr. Because of antigenic heterogeneity of the determinant, there are 10 major serotypes of Hepatitis B virus.
The HBsAg RapiCard™ is a rapid test to qualitatively detect the presence of HBsAg in whole blood,serum or plasma specimen. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood,serum or plasma.

TEST PRINCIPLE

The HBsAg RapiCard™ is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in whole blood,serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the cassette. During testing, the whole blood,serum or plasma specimen reacts with the particle coated with anti-HBsAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. 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.

SPECIMEN COLLECTION AND PREPARATION

- The HBsAg RapiCard™ can be performed using whole blood (from venipuncture or fingerstick),serum or plasma.
- To collect **Fingerstick Whole Blood specimens**:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  - Add the Fingerstick Whole Blood specimen to the test by using **a capillary tube**:
    - Touch the end of the capillary tube to the blood until filled to approximately 75 µL. Avoid air bubbles.
    - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
  - Add the Fingerstick Whole Blood specimen to the test by using **hanging drops**:
    - Position the patient's finger so that the drop of blood is just above the specimen area of the test cassette.
    - Allow 3 hanging drops of fingerstick whole blood to fall into the center of the specimen area on the test cassette, or move the patient's finger so that the hanging drop touches the center of the specimen area. Avoid touching the finger directly to the specimen area.
- 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 the specimens have been collected. 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. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- 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 covering the transportation of etiologic agents.

REAGENTS

The test Cassette contains anti-HBsAg particles and anti-HBsAg coated on the membrane.

Materials provided

- Test cassettes
- Droppers
- Buffer
- Package insert

Materials required but not provided

- Specimen collection containers
- Centrifuge
- Lancets (for fingerstick whole blood only)
- Timer
- Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

PRECAUTION

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

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1. For professional in vitro diagnostic use only. Do not use after the expiration date.
2. The test should remain in the sealed pouch or closed canister until ready to use.
3. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
4. The used test should be discarded according to local regulations.

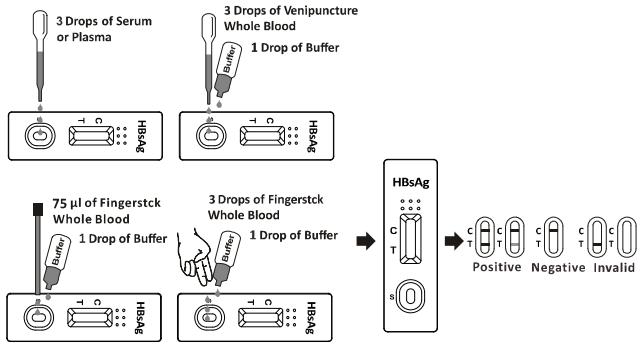
ASSAY PROCEDURE

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the cassette on a clean and level surface.
   For **Serum or Plasma** specimen:
   - Hold the dropper vertically and transfer **3 drops of serum or plasma** (approximately 75 µL) to the specimen well of test Cassette and start the timer. See illustration below.

   For **Venipuncture Whole Blood** specimen:
   - Hold the dropper vertically and transfer **3 drops of whole blood** (approximately 75 µL) to the specimen area, then **add 1 drop of buffer** (approximately 40 µL), and start the timer. See illustration below.

   For **Fingerstick Whole Blood** specimen:
   - To use a capillary tube: Fill the capillary tube and **transfer approximately 75µL of fingerstick whole blood specimen** to the specimen area of test cassette, then add **1 drop of buffer (approximately 40 µL)** and start the timer. See illustration below.
   - To use hanging drops: Allow **3 hanging drops of fingerstick whole blood s**pecimen (approximately 75 µL) to fall into the specimen area of test cassette, then add **1 drop of buffer (approximately 40 µL) a**nd start the timer. See illustration below.
3. Wait for the colored line(s) to appear. **Read results at 15 ~30 minutes.** Do not interpret the result after 30 minutes.



RESULTS

(Please refer to the illustration above)
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 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.

QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control 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 a positive control (containing 10ng/mL HBsAg) and a negative control control (containing 0 ng/mL HBsAg) be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

STORAGE

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

LIMITATIONS

1. The HBsAg RapiCard™ is for professional in vitro diagnostic use only. The test should be used for the detection of HBsAg in whole blood,serum or plasma specimen. Neither the quantitative value nor the rate of HBsAg concentration can be determined by this qualitative test.
2. The HBsAg RapiCard™ will only indicate the presence of HBsAg 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.
4. The HBsAg RapiCard™ cannot detect less than 1 PEI ng/ml of HBsAg in specimens. 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 infection.

EXPECTED VALUES

The HBsAg RapiCard™ (Whole Blood/Serum/Plasma) has been compared with a leading commercial HBsAg EIA test. The correlation between these two systems is over 99%.

PERFORMANCE CHARACTERISTICS

Sensitivity

The HBsAg RapiCard™ (Whole Blood/Serum/Plasma) was tested against a sensitivity panel including both ad and ay subtypes with concentrations ranging from 0 to 300ng/ml. The test can detect 1 PEI ng/ml of HBsAg in whole blood, serum or plasma.

Specificity

Antibodies used for the HBsAg RapiCard™ (Whole/Blood/Serum/Plasma) were developed against whole Hepatitis B antigen isolated from Hepatitis B virus. Specificity of the HBsAg RapiCard™ (Whole Blood/Serum/Plasma) was also

tested with laboratory strains of Hepatitis A and Hepatitis C. They all yielded negative results.

Serum or plasma specimens:

Method		ELISA		Total Results
HBsAg RapiCard™ (WB/Serum/Plasma)	Result	Positive	Negative	
	Positive	180	2	182
	Negative	0	550	550
		180	552	732

Relative Sensitivity: > 99.9% (95%CI:*98.3%-100%)
Relative Specificity: 99.6% (95%CI:*98.7%-99.9%)
Overall accuracy: 99.7% (95%CI:*99.0%-99.9%)
*Confidence Intervals

Whole blood specimens:

Method		ELISA		Total Results
HBsAg RapiCard™ (WB/Serum/Plasma)	Result	Positive	Negative	
	Positive	180	1	181
	Negative	0	200	200
		180	201	381

Relative Sensitivity: > 99.9% (95%CI:*98.3%-100%)
Relative Specificity: 99.5% (95%CI:*97.3%-99.9%)
Overall accuracy: 99.7% (95%CI:*98.5%-99.9%)
*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of six specimens containing 0ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 12ng/ml and 20ng/ml of HBsAg. The negative and positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by using the same six specimens of 0ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 12ng/ml and 20ng/ml of HBsAg in 3 independent assays. Three different lots of the HBsAg RapiCard™ (Whole Blood/Serum/Plasma) have been tested using negative, low positive and high positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

Specimens positive for HAMA, and Rheumatoid factor (RF) have been tested. No cross-reactivity was observed, indicating that the HIV, SYP, HCV and HAV.

Interfering Substances

The HBsAg RapiCard™ (Whole Blood/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.

REFERENCE

1. Blumberg, B.S. The Discovery of Australian Antigen and its relation to viral hepatitis. Vitro.1971; 7: 223
2. World Health Organization. HEPATITIS B SURFACE ANTIGEN ASSAYS: OPERATIONAL CHARACTERISTICS (PHASE I) report 1. 2001; 2-4

ISO 13485
ISO 9001

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Date Adopted	**2017-03-29**
REF 176571-1-44	**CORTEZ- OneStep HBsAg RapiCard™ InstaTest (Serum/Plasma/WB)**
Revision Date: 2016-12-27	