HEPATITIS B SURFACE ANTIGEN (HBsAg) RAPID TEST
(Whole blood/Serum/Plasma - Cassette)
REF: CHB-203

INTENDED USE

HBsAg Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in whole blood, serum or plasma. This test is for screening of Hepatitis B Surface Antigen and it is for Professional use Only.

INTRODUCTION

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease.

The virus is transmitted through contact with the blood or other body fluids of an infected person.

An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive).

In 2015, hepatitis B resulted in 887 000 deaths, mostly from complications (including cirrhosis and hepatocellular carcinoma).

Hepatitis B is an important occupational hazard for health workers.

However, it can be prevented by currently available safe and effective vaccine.

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer.

Laboratory diagnosis of hepatitis B infection focuses on the detection of the hepatitis B surface antigen HBsAg. WHO recommends that all blood donations be tested for hepatitis B to ensure blood safety and avoid accidental transmission to people who receive blood products.

- Acute HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) antibody to the core antigen, HBcAg. During the initial phase of infection, patients are also seropositive for hepatitis B e antigen (HBeAg). HBeAg is usually a marker of high levels of replication of the virus. The presence of HBeAg indicates that the blood and body fluids of the infected individual are highly infectious.
- Chronic infection is characterized by the persistence of HBsAg for at least 6 months (with or without concurrent HBeAg). Persistence of HBsAg is the principal marker of risk for developing chronic liver disease and liver cancer (hepatocellular carcinoma) later in life.

HBsAg, an HBV viral coat antigen is produced in large quantities in infected-cell cytoplasm and continues to be produced in patients with chronic, active HBV infection. This has for many years been used as the primary screening test. HBsAg can be identified in an infected donors' serum or plasma by enzyme immunoassays (EIA) using animal antibodies (anti-HBs) as the solid-phase capture reagent and conjugated anti-HBs as the probe.

The test is used to identify those at risk of spreading the disease, e.g., blood donors, pregnant women, intravenous drug abusers, healthcare workers, institutionalized people, transplant donors and recipients, and donors of semen for artificial insemination.

HBsAg screening assays are normally supported by confirmatory tests, which are used to confirm repeatable reactive (positive) results.

PRINCIPLE

HBsAg Rapid Test Cassette 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 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 HBsAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with 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.

PRODUCT CONTENTS

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

MATERIALS SUPPLIED

1.Test cassette 2.Dropper 3.Buffer 4. One Instructions for use

MATERIAL REQUIRED BUT NOT PROVIDED

1.Specimen collection containers 2.Centrifuge 3.Timer

For fingerstick whole blood only:

1.Lancet 2.Heparinized capillary tubes and dispensing bulb

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. The test must remain in the sealed pouch until use. Do not freeze. Do not use beyond the expiration date.

STABILITY

Tests are stable for 24 months when stored 2-30 ^{0}C conditions.

Tests are stable for 1 hour after aluminum pouch is opened when stored 2-30 ^{0}C conditions.

WARNINGS AND PRECAUTIONS

- All components included in the box are intended for "in vitro diagnostic use" for Professional usage.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled and tests are being performed.
- Do not pipette by mouth.
- Any equipment directly in contact with specimens should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples. Wipe spills immediately and decontaminate affected surfaces.
- Provide adequate ventilation.
- Warning-potential biohazards material: all blood derivatives should be considered potentially infectious, it is recommended that these specimens be handled and safe disposed as medical waste using established good laboratory working practices.
- Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste.
- Do not use test kit beyond expiry date.
- The test device should never be reused.
- Use a fresh transfer pipette for each whole blood, serum or plasma specimen.
- The HBsAg device should remain in its original sealed pouch until ready for use. Do not use the test if the seal is broken or the pouch is damaged.
- Turbid test samples should be centrifuged.
- Frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.
- As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.
- The reliability of the results depends on correct implementation of the following Good laboratory Practices.
- Avoid exposure of the tests to excessive heat or sunlight during storage.
- Do not change the assay procedure.
- Physicians or medical technicians only should handle this reagents kit.
- An ID space is provided at device design, for specimen identification usage.

COLLECTION AND HANDLING OF SAMPLE

- HBsAg Rapid Test Cassette can be performed using whole blood (from venipuncture or fingerstick),serum or plasma.
- To collect **Fingerstick Whole Blood specimens**:
  1. Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  2. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  3. Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  4. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  5. 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 50 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.
  6. 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 2 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.

TEST PROCEDURE

Allow the test, specimen, buffer and/or controls to reach room temperature prior to testing.

1. Bring the pouch to room temperature before opening it.
2. Remove the test cassette from the sealed pouch and use it as soon as possible.
3. 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(S) of test Cassette and start the timer. See illustration below.

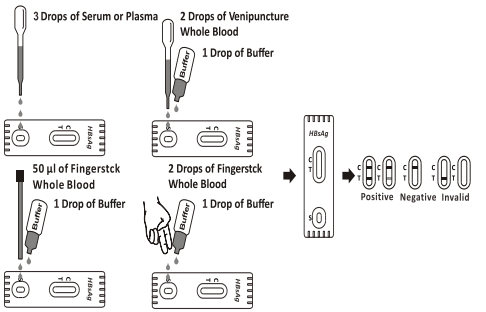
For **Venipuncture Whole Blood** specimen:

- Hold the dropper vertically and transfer **2 drops of whole blood** (approximately 50 μL) to the specimen area (S), 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 50μL of fingerstick whole blood specimen** to the specimen area(S) of test cassette, then add **1 drop of buffer (approximately 40 μL)** and start the timer. See illustration below.
- To use hanging drops: Allow **2 hanging drops of fingerstick whole blood s**pecimen (approximately 50 μL) to fall into the specimen area(S) of test cassette, then add **1 drop of buffer (approximately 40 μL) a**nd start the timer. See illustration below.

4. Wait for the colored line(s) to appear. **Read results at 15 ~30 minutes.** Do not interpret the result after 30 minutes.



INTERPRETATION OF RESULTS

POSITIVE:* Two distinct red 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 red colored line appears in the control region (C). No apparent red 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 10 ng/mL HBsAg) and a negative control (containing 0 ng/mL HBsAg) be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

RESTRICTIONS

- Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
- As in all sensitive immunoassay, there is the possibility that false positive results occur.
- A negative result does not exclude the possibility of exposure or infection with HBV.
- The kit is a qualitative assay, and can not be used as a quantitative assay.
- This kit is only used for the detection of human whole blood/serum/plasma samples.
- Samples with positive result should be further investigated with other methods. Repeatedly positive samples should be retested with an additional confirmatory assay.
- Although there is a close association between HBsAg and the level of infectivity, currently available methods cannot detect and identify all infected blood samples or cases of HBV infection.
- Interpretation of a reactive result should not be based only on the result of the screening test. Repeatably positive samples should be retested with a confirmatory test to establish the specificity of the result.
- The HbsAg test cannot detect extremely low concentrations of HBsAg in specimens. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is required. A negative result at any time does not preclude the possibility of Hepatitis B infection.
- This test will indicate only the presence or absence of HbsAg in the specimen, and should not be used as the only basis for the diagnosis of Hepatitis viral infection. As with all diagnostic tests, results must be considered with other clinical information available to the physician.
- Performance results and percentages are based on the run studies, there may show vary when any other study is conducted.
- The device is not intended for handicapped persons, children or elderly.
- New drugs, biochemical metabolites, heterophilic antibodies and sample preparation materials can affect the performance characteristics.

PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications (CTS) as required by art. 5, Chapter 3 of IVD Directive 98/79/EC).

SENSITIVITY

The diagnostic sensitivity of the HBsAg Rapid Test was based on testing of a panel of 403 HBsAg positive samples, 30 seroconversion panels.

In details:

- 403 Positive Samples including subtypes; ad, ay, adr, adw, ayw. All positive samples are detected as positive with HBsAg Rapid Test.

Issued Date &Revision May 2018/0 COM.RIFU.20 CKS-114

Actual Status	CHIL HBsAg Rapid Test Results		
	Positive	Negative	Total
Negative	0	0	0
Positive	401	2	403
Total	401	2	403

A final sensitivity has been found 99.50%.

- 30 Seroconversion Panels are tested and results were shown early or equal or late seroconversion detection when comparing with other CE-marked assays.

ANALYTICAL SENSITIVITY (Limit of Detection)

The analytical sensitivity was determined by means of the WHO international standard for HBsAg, Third International Standard for HBsAg (HBV genotype B4, HBsAg Subtypes ayw1/adw2) NIBSC code 12/226.

The Analytical Sensitivity (Limit of Detection) has been estimated < 0,50 IU/mL.

SPECIFICITY

The specificity evaluated on below samples:

- 1057 Unselected blood donors includind first time donors

From the 1057 unselected blood donors, 7 samples were positive and tested in duplicate. These resulted in 6 samples repeatedly positive and the rest 1050 samples were negative both by reference assay and CHIL HBsAg Rapid Test which results are demonstrated below:

Actual Status	CHIL HBsAg Rapid Test Results		Total
	Positive	Negative	
Negative	1	1050	1051
Positive	6	0	6
Total	7	1050	1057

A final specificity has been found 99.91%.

- 203 Hospitalized patients
- 201 Pregnancy samples
- 101 Potentially interfering samples:
  - ✓ 30 Rheumatoid Factor (RF) positive ssamples
  - ✓ 12 Hemolyzed samples including heavy hemolyzed
  - ✓ 6 Bilirubin samples
  - ✓ 18 HCVAb positive samples
  - ✓ 15 HIVAb positive samples
  - ✓ 6 EBV IgG positive samples
  - ✓ 6 HSV 1 IgG positive samples
  - ✓ 4 Toxoplasmosis IgG positive samples
  - ✓ 4 Rubella IgG positive samples

All negative samples are tested as negative with HBsAg Rapid Test.

- 50 Samples (25 Positive and 25 Negative) have been tested and no difference due to the method for sample preparation (Citrate, EDTA and Heparin Anticoagulants) has been observed.
- In all studies whole blood, serum and plasma types are used and there is no difference on results due to sample type observed.

PRECISION

Inter Lot Precision

Negative and positive samples which confirmed by Reference Assay were used for inter lot precision. Lot was checked with 4 negative and 4 positive samples by 20 times.

%Agreement is found as %100 for Negative samples.

%Agreement is found as %98,75 for Positive samples.

Intra Lot Precision

Negative and positive samples which confirmed by Reference Assay were used for intra lot precision. Three lots were checked with 3 negative and 3 positive samples under same conditions.

%Agreement is found as %100 for Negative samples.

%Agreement is found as %98,89 for Positive samples.

REFERENCE

1. http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-b, 5 July 2017
2. HBsAg is the serologic hallmark of HBV infection and is the first marker to appear after acute infection. From: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015 https://www.sciencedirect.com/topics/immunology-and-microbiology/hbsag
3. Ravi Kaul, in The Immunoassay Handbook (Fourth Edition), 2013
4. Debra A. Kessler RN, MS, in Transfusion Medicine and Hemostasis (Second Edition), 2013

CHIL TIBBİ MAL. SAN. TİC. LTD. ŞTİ.
10028 sok. No.11 AOSB 35620 Cigli-Izmir/Turkey
Tel:+90 232 2901688, Fax: +90 232 2901323
E-mail: info@chil.com.tr www.chil.com.tr

CE 1434	CE marking		Manufacturer	LOT	Lot code
IVD	For in vitro diagnostic use		Expiry date		Biological Risk
2°C 30°C	Storage temperature limitation		Consult Instruction for use		Keep Away from Sunlight
Σ	Test per kit		Do not re-use		Keep Dry
REF	Catalogue Number				