SPECTRUM

The Creative Approach To Bioscience

# HBsAg/HCV Ab Rapid Test - Cassette
**(Serum/Plasma/Whole Blood)**

REF: 1162 001 25 test
REF: 1162 002 50 test

## INTENDED USE
The Spectrum HBsAg/HCV Ab Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus antibodies (IgG, IgM, IgA) in human serum, plasma and whole blood. It is intended to be used as a screening test and as an aid in the diagnosis of infection with Hepatitis B virus (HBV) and Hepatitis C virus (HCV). Any reactive specimen with the Spectrum HBsAg/HCV Ab Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

## SUMMARY AND EXPLANATION OF THE TEST
Hepatitis B virus (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Western countries) to 20% (Asia, Africa)1.

Hepatitis C, caused by Hepatitis C virus (HCV) infection, was formerly described as the parenterally transmitted form of non-A, non-B hepatitis (NANBH). HCV can also be transmitted through intravenous drug abuse, sexual, and household contact. About 3% of population is infected with HCV3, 15% of cases of infection are acute and 80% of infections become chronic disease and are often associated with cirrhosis and hepatocellular carcinoma4. Cirrhosis is more common in those co-infected with Hepatitis B virus.

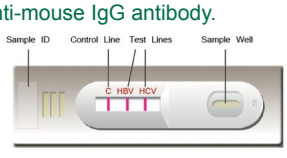
Dual infection with HBV and HCV is not uncommon in geographic areas where a high endemic level of both infections is reported such as Southeast-Asia and the Mediterranean. In general, the prevalence is around 10-20% in patients with chronic HBV infection, and 2-10% of anti-HCV-positive patients have markers of HBV infection. Co-infection of HBV and HCV was found to be high in HIV-infected people (66%), particularly in HIV infected drug users (84%) 5, 6.

Hepatitis B surface antigen (HBsAg) is the first marker to appear in the blood in acute Hepatitis B, detected 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms7. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups8.

Hepatitis C antibody (HCV Ab) tests such as ELISA and chromatographic immunoassay are the most common tests for detection of HCV infection as detectable levels of HCV Ab are generated about 6–8 weeks following infection 9-12.

The Spectrum HBsAg/HCV Ab Rapid Test is a point of care test which can detect both HBsAg and HCV antibodies in serum, plasma or whole blood in one device in 15 minutes by personnel with minimal training and without laboratory equipment.

## TEST PRINCIPLE
TThe Spectrum HBsAg/HCV Ab Rapid Test is a 3 line, lateral flow chromatographic immunoassay based on antibody sandwich assay (for HBsAg) and double antigen assay (for HCV Ab) test principles. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant HCV core and non-structure antigens conjugated with colloidal gold (HCV Ag conjugates) and mouse anti-HBsAg antibody conjugated with colloidal gold (anti-HBsAg conjugates), 2) a nitrocellulose membrane strip containing two test bands (HCV and HBV bands) and a control band (C band). The HCV band is pre-coated with recombinant HCV core and non-structure antigens, the HBV band is pre-coated with non-conjugated HBsAg antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.




### SYMBOLS IN PRODUCT LABELLING

| | | | |
|---|---|---|---|
| EC REP | Authorised Representative | | Temperature Limitation |
| IVD | For in-vitro diagnostic use | | Use by/Expiration Date |
| LOT | Batch Code/Lot number | | CAUTION. Consult instructions for use |
| REF | Catalogue Number | | |
| | Consult instructions for use | | Manufactured by |

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. The antibodies to HCV, if present in the specimen, will bind to the HCV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated non-conjugated HCV antigens on the HCV band forming a burgundy colored HCV band, indicating a HCV Ab positive or reactive test result.

HBsAg if present in the specimen will bind to the anti-HBsAg conjugates. The immunocomplex is then captured on the membrane by the pre-coated non-conjugated HBsAg antibody on the HBV band forming a burgundy colored HBV band, indicating a HBsAg positive test result.

Absence of test bands suggests negative results. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG/anti-HBsAg conjugate regardless of the presence of colored test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

## REAGENTS AND MATERIALS PROVIDED
1. Individually sealed foil pouches containing::
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Sample Diluent (1 vial, 5 mL)
4. One package insert (instruction for use)

## MATERIALS MAY BE REQUIRED AND NOT PROVIDED
1. Positive Control
2. Negative Control

## MATERIALS REQUIRED BUT NOT PROVIDED
1. Clock or Timer
2. Lancing device

## WARNINGS AND PRECAUTIONS
***For in Vitro Diagnostic Use***

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

## REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C. Do not expose the kit over 30°C. Do not freeze the kit. The negative and positive controls should be store at 2-8°C or the temperature indicated. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch.

## SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

**Plasma**

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer® ) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

**Serum**

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C to 8°C if not tested immediately. Store specimens at 2°C to 8°C up to 5 days. The specimens should be frozen at -20°C for longer storage

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

**Blood**

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Do not use any hemolized blood for testing.

Whole blood specimens should be stored in refrigeration (2°C-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

## ASSAY PROCEDURE

**Step 1:** Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

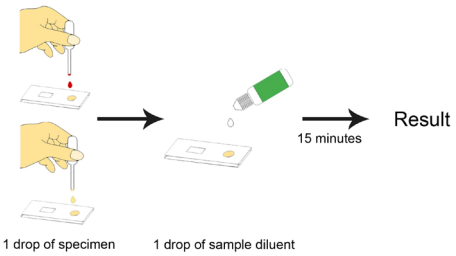
**Step 2:** When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

**Step 3:** Be sure to label the device with specimen's ID number.

**Step 4:** Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop of specimen (about 40-50 µL for whole blood, 30-45 µL for serum/plasma) into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 µL) of Sample Diluent.




***Step 5:*** Set up timer.

***Step 6:*** Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

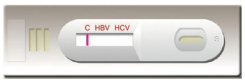
***Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.***

## QUALITY CONTROL

1. ***Internal Control:*** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.
2. ***External Control:*** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kits fall outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

## INTERPRETATION OF ASSAY RESULT

1. ***NEGATIVE RESULT:*** If only the C band is developed, the test indicates that the levels of anti-HCV antibodies and HBsAg in the specimen are undetectable. The result is negative or non-reactive.



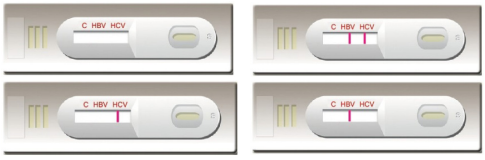

2. ***POSITIVE RESULT:***
   I. If both C and HCV bands are developed, the test indicates the presence of antibodies to HCV in the specimen. The result is HCV antibody positive.


   II. If both C and HBV bands are developed, the test indicates that the specimen contains HBsAg. The result is HBsAg positive.


   III. If C band, HCV band and HBV band are all developed, the test indicates the presence of antibodies to HCV and HBsAg. The result is HCV antibody positive and HBsAg positive.


Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. ***INVALID:*** If no C line is developed, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.




## PERFORMANCE CHARACTERISTICS

1. ***Clinical Performance***

A total of 1050 samples from susceptible subjects were tested with the Spectrum HBsAg/HCV Ab Rapid Test and by a commercial HCV Ab ELISA kit. Comparison for all subjects is shown in the following table

| | ***Spectrum HBsAg/HCV Ab Rapid Test*** | | |
|---|---|---|---|
| HCV Ab ELISA | Positive | Negative | Total |
| Positive | 312 | 4 | 316 |
| Negative | 3 | 731 | 734 |
| Total | 315 | 735 | 1050 |

HCV Ab Relative Sensitivity: 98.7%, Relative Specificity: 99.6%, Overall Agreement: 99.3%

A total of 1056 samples from susceptible subjects were tested with the Spectrum HBsAg/HCV Ab Rapid Test and by a commercial HBsAg ELISA kit. Comparison for all subjects is shown in the following table.

| | Spectrum HBsAg/HCV Ab Rapid Test | | |
|---|---|---|---|
| HBsAg ELISA | Positive | Negative | Total |
| Positive | 322 | 0 | 322 |
| Negative | 2 | 732 | 734 |
| Total | 324 | 732 | 1056 |

HBsAg relative Sensitivity: 100%, Relative Specificity: 99.7%, Overall Agreement: 99.8%

***2. Cross-Reactivity***

Cross-reactivity with specimens from other infectious diseases: Specimens from other common infectious diseases were collected and tested with the Spectrum HBsAg/HCV Ab Rapid Test for a cross reaction study. The result is shown in the table below:

| Specimens | Sample size | HCV Ab Reactivity | HBsAg Reactivity |
|---|---|---|---|
| HAV positive serum | 10 | Negative | Negative |
| HIV positive serum | 10 | Negative | Negative |
| HBsAg positive serum | 10 | Negative | Positive |
| HCV Ab positive serum | 10 | Positive | Negative |
| Syphilis positive serum | 10 | Negative | Negative |
| TB positive serum | 10 | Negative | Negative |
| H. pylori positive serum | 10 | Negative | Negative |
| ANA serum | 8 | Negative | Negative |
| RF (≤2,500 IU/ml) serum | 3 | Negative | Negative |
| HAMA specimens | 19 | Negative | Negative |

***Cross reaction with common microbe antigens:***

The negative blood specimen was spiked with antigens from common microbes and then tested according to the standard procedure. The results showed that the Spectrum HBsAg/HCV Ab Rapid Test had no cross-reaction with the following antigens at the concentration tested.

| Antigen (Ag) | Concentration spiked | HCV Ab Reactivity | HBsAg Reactivity |
|---|---|---|---|
| HIV P24 Ag | 1.0 mg/mL | Negative | Negative |
| Dengue virus NS1 Ag (I, II, III,IV) | 1.0 mg/mL | Negative | Negative |
| Chikungunya Ag | 1.0 mg/ml | Negative | Negative |

***3. Interference***

Common substances (such as pain and fever medication, blood components) may affect the performance of the Spectrum HBsAg/HCV Ab Rapid Test. This was studied by spiking of these substances to the three levels of the HCV Ab and HBsAg. The results are presented on the following table and demonstrate that the substances studied did not affect the performance of the Spectrum HBsAg/HCV Ab Rapid Test.

Note: -: Negative; +: Weak Positive; +++: Strong Positive

| Potential interfering substances spiked | HCV Ab Reactivity | | | HBsAg Reactivity | | |
|---|---|---|---|---|---|---|
| | Negative | Weak Positive | Strong Positive | Negative | Weak Positive | Strong Positive |
| Control | - | + | +++ | - | + | +++ |
| Bilirubin 20 mg/dL | - | + | +++ | - | + | +++ |
| Creatinine 442 µmol/L | - | + | +++ | - | + | +++ |
| Glucose 55 mmol/L | - | + | +++ | - | + | +++ |
| Albumin 60 g/L | - | + | +++ | - | + | +++ |
| Salicylic acid 4.34 mmol/L | - | + | +++ | - | + | +++ |
| Heparin 3,000 U/L | - | + | +++ | - | + | +++ |
| EDTA 3.4 µmol/L | - | + | +++ | - | + | +++ |
| Human IgG 150 mg/dL | - | + | +++ | - | + | +++ |

## LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing for the presence of anti-HCV antibodies and/or HBsAg in serum, plasma and whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Spectrum HBsAg/HCV Ab Rapid Test is limited to the qualitative detection of anti HCV antibodies and/or HBsAg in human serum, plasma and whole blood. The intensity of the test bands does not have linear correlation with anti-HCV antibodies titer or HBsAg titer in the specimen.
3. A nonreactive test result does not preclude the possibility of exposure to or infection with HCV and/or HBV.
4. A nonreactive result can occur if the quantity of anti-HCV antibody and/or HBsAg present in the specimen is below the detection limits of the assay, or anti-HCV antibody and/or the HBsAg that are detected are not present during the stage of disease in which a sample is collected.
5. If symptoms persist, while the result from Spectrum HBsAg/HCV Ab Rapid Test is non-reactive, it is recommended to re-sample the patient or test with an alternative test method.
6. Some specimens containing an unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

## REFERENCES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