First Response® HCV Card Test is a chromatographic immunoassay for qualitative detection of the antibodies against hepatitis C virus (HCV Ab) in human serum, plasma or whole blood samples. It is intended for use in medical institution as an aid for diagnosis and management of patients related to infection with hepatitis C as well for primary screening of blood from volunteer donors on the spot.

**Assay Principle**

First Response® HCV Card Test employs chromatographic lateral flow device in a cassette format. Colloidal gold conjugated goat anti-human IgM and mouse anti-human IgG are dried and immobilized on the fiberglass strip. HCV antigens are immobilized at the Test Zone (T) and goat anti mouse IgG antibodies are immobilized at the Control Zone (C). When the sample is added, it migrates by capillary diffusion rehydrating the gold conjugate. If present in sample, HCV antibodies will bind the gold conjugated anti-human IgG and/or IgM forming complexes. These complexes will continue to migrate along the strip until the Test Zone (T) zone where they are captured by the HCV antigens to form a visible red line. The un-bound gold conjugate will continue to move and bind with goat antimouse IgG at the Control Zone (C) forming a visible red line. If no HCV antibodies in sample, only a red line is appeared at the Control Zone (C), which indicates the validity of the test.

**Intended Use**

First Response® HCV Card Test is a chromatographic immunoassay for qualitative detection of the antibodies against hepatitis C virus (HCV Ab) in human serum, plasma or whole blood samples. It is intended for use in medical institution as an aid for diagnosis and management of patients related to infection with hepatitis C as well for primary screening of blood from volunteer donors on the spot.

**Introduction**

Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form. More than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced. Clinical studies show that significant amount of HCV infected individuals develop antibodies to NS5 nonstructural protein of the virus. For this, the third generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Third generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies (window period) to 60 days.

**Materials Provided**

First Response® HCV Card Test kit contains the following items to perform the assay.

- **Reagents**
  - Test Device with sample pipette and desiccant (Test device enclosed nitrocellulose test strip on which test and control lines are coated).
  - Assay Diluent (Carbonate buffer saline containing process and preservative).

- **Accessories**
  - Instructions for use
  - Alcohol Swab
  - Sterile lancet

- **Materials Required but Not Provided**: Timer

**Storage & Stability**

First Response® HCV Card Test should be stored at 4 - 30°C. Do not freeze the kit or components. Assay diluent (opened & unopened) & the unopened Test Device are stable until the expiry date printed on the label, when stored at room temperature 4 - 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the Test Device from the foil pouch. The shelf life of the kit is as indicated on the outer package. Do not use Test Device and Assay Diluent beyond the date of expiry.

**References**

Precautions

1. For in vitro diagnostic use only.
2. Test Devices and Assay diluent of different lot must not be used.
3. Do not use the Test Device if the pouch is not intact.
4. Do not use the Lancet if the seal is broken.
5. Check the desiccant for saturation, immediately after opening the pouch.
6. Do not smoke, eat or drink while handling specimens and performing a test.
7. The test device, alcohol swab, lancet and sample pipette are intended for single use only.
8. Follow the assay procedure strictly, deviation will invalidate the results.
9. Perform the test by using kit assay diluent, any other diluent or fluid will invalidate the results.
10. Do not touch the tip of Assay Diluent bottle, it might contaminate Assay Diluent.
11. Wear protective gloves while handling specimens. Dispose off used gloves as biohazard waste. Wash hands thoroughly afterwards.
12. Avoid splashing or aerosol formation.
13. Clean up spills thoroughly using an appropriate disinfectant.
14. Decontaminate and dispose off all used specimens, test devices, alcohol swabs, lancets and sample pipette as a biohazard waste.

Specimen Collection and Storage

1. Capillary blood collection: Clean the area to be lanced with an alcohol swab and allow it to air dry. Squeeze the end of the fingertip and pierce with a sterile lancet provided. Inser the open end in the blood drop and then release the pressure to draw blood into the blood drop.
2. Venous Blood collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture.
3. Plasma collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture. Centrifuge the tube to obtain Plasma.
4. Serum: Collect Serum in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture. Centrifuge the tube to obtain Plasma.
5. Whole Blood specimen may be used for testing immediately or may be stored at 2-8°C for upto 3 days.
6. If Serum or Plasma specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than three days, freezing at -20°C is recommended. They should be brought to room temperature prior to use.
7. Serum or Plasma specimens containing precipitate may yield inconsistent test results. Such specimens must ALWAYS be centrifuged prior to assaying.

Test Procedure

1. Bring the First Response® HCV Card Test kit components to room temperature prior to testing.
2. Remove the Test Device and the sample pipette from the foil pouch and place it on a flat, dry surface.
3. Slowly add 35 μl (one drop) of Whole Blood / serum / plasma to the sample well (S) using the Sample Pipette. Dispose off used sample pipette as biohazard waste.
4. Add 35 μl (one drop) of the Assay Diluent to the sample well (S).
5. Observe developments of colored bands in the Results Window.
6. Test procedure results at 20 minutes. After recording the results, dispose off test device as a biohazard waste.
7. Do not interpret after 30 minutes.

Interpretation of the Test

Negative Result

If only one color band appear, at control line 'C' as in the figure, the specimen is negative.

Positive Results

If two color bands appears, one at control line 'C' and other at test line 'T' as in the figure, the specimen is reactive for antibodies to HCV.

Invalid Result

If no color band appears at the control line 'C' within the stipulated time then the result is invalid.

Limitations

1. The anti-coagulants such as heparin, EDTA and citrate do not affect the test result.
2. Hemolytic sample may give reddish background even after end of test time.
3. Repeat the test in case of very faint test line or if have any doubt for test line.
4. False negative results may arise because of hook effect due to very high titre of antibody in sample. Repeat the test by using different dilution of same sample.
5. Negative results do not rule out the possibility of hepatitis C exposure or infection. Infection through recent exposure to HCV may not be detectable.
6. The positive result obtained with First Response® HCV Card Test alone cannot be the final diagnosis of hepatitis C infection. Any positive result must be interpreted in conjunction with the patient clinical history and another laboratory testing results. Follow-up and supplementary testing with other analytical system (e.g. ELISA) is required to confirm any positive results.
7. First Response® HCV Card Test is intended ONLY for testing of individual whole blood / serum / plasma sample. DO NOT use it for testing of other body fluids or pooled blood samples.
8. This is a qualitative assay and the results cannot be used to measure antibody concentrations.

Performance Characteristics

(A) 1. First Response® HCV Card Test have been tested using an in-house panel of Positive and Negative clinical samples confirmed by a leading commercial anti-HCV ELISA kit. The result shows that First Response® HCV Card Test is very accurate compared to other commercial ELISA kit. In a comparison of the First Response® HCV Card Test versus a leading commercial anti-HCV ELISA and Rapid test, results gave sensitivity of 99.75%, A specificity of 99.50% and total agreement of 99.62%.

(B) Precision

2. (a) Within run precision was determined by using 10 replicates of four different specimens containing different concentrations of antibodies. Within run precision was observed 100%.

2. (b) Between run precision was determined by using the four different specimens containing different concentrations of antibody in 3 different replicates with 3 different lots of test devices. Between run precision was observed 100%.