HCV (HEPATITIS C VIRUS) RAPID TEST
(Whole blood/serum/Plasma – Cassette)
REF: CHC-203

INTENDED USE

HCV Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of antibody to Hepatitis C Virus in whole blood or serum or plasma.

INTRODUCTION

Hepatitis C is a liver disease caused by the hepatitis C virus: the virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few weeks to a serious, lifelong illness.

The hepatitis C virus is a bloodborne virus and the most common modes of infection are through exposure to small quantities of blood. This may happen through injection drug use, unsafe injection practices, unsafe health care, and the transfusion of unscreened blood and blood products.

Globally, an estimated 71 million people have chronic hepatitis C infection.

A significant number of those who are chronically infected will develop cirrhosis or liver cancer.

Approximately 399 000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma.

Serological assays for detecting anti-HCV were developed and improved following the initial discovery of the virus because of the urgent need to screen blood donors and prevent transmission. Anti-HCV is typically identified by using enzyme-linked immunosorbent assay (ELISA). Three generations of ELISAs have been developed since 1989. The first generation assays, which incorporated the recombinant c100-3 epitope from the NS4 region, were used until 1992, when they were replaced by second generation assays, which additionally incorporated epitopes c22-3 and c33c from the HCV core and NS3 regions, respectively. The third generation assays contained reconfigured core and NS3 antigens and in addition a newly incorporated antigen from the NS5 region.

The window period has been documented to decrease from approximately 16 weeks to 10 weeks and finally to 8 weeks with the introduction of first-, second-, and third-generation anti-HCV ELISAs, respectively. The newest generation of immunoassays available, that is, fourth generation of tests is those that simultaneously detect HCV capsid antigen as well as antibodies to the core, NS3, NS4, and NS5 regions of the virus. These assays have further reduced the window period of HCV detection by 17 days to already existing assays. But the literature supporting the inclusion of these assays as 4th generation on the basis of improved sensitivity, specificity is limited.

PRINCIPLE

HCV Rapid Test Cassette is a qualitative, membrane based immunoassay for the detection of antibody to HCV in whole blood or serum or plasma. The membrane is pre-coated with recombinant HCV antigen on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with recombinant HCV antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

PRODUCT CONTENTS

The test cassette contains recombinant HCV antigen conjugated colloid gold and HCV antigen coated on the membrane.

MATERIALS SUPPLIED

1. Test cassettes 2. Droppers 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. Lancets 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 infectious 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 material is 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 HCV 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

- HCV Rapid Test Cassette can be performed using whole blood, 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 new 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 device 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 into the specimen well of the test device.
  6. Add the Fingerstick whole blood specimen to the test device by using hanging drops:
     - Position the patient's finger so that the drop of blood is just above the specimen well of the test device.
     - Allow 2 hanging drops of fingerstick whole blood to fall into the center of specimen well on the test device, or move the patient's finger so that the hanging drop touches the center of the specimen well. Avoid touching the finger directly to the specimen well.
- 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

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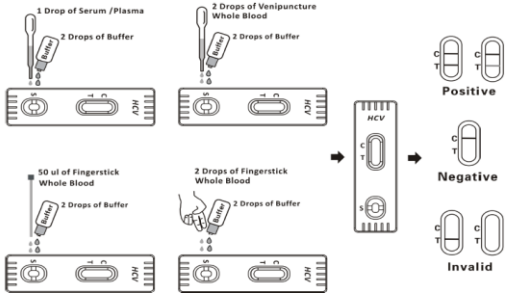
plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick 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 test cassette, specimen, and/or controls to equilibrate to room temperature prior to testing.

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 immediately after opening the foil pouch.
2. **For Serum or Plasma specimen**: Hold the dropper vertically and transfer 1 drop of serum or plasma (~25 µL) to the specimen well(S) of test device, then add 2 drops of buffer (~80 µL) and start the timer. See illustration below.
3. **For Venipuncture Whole Blood specimen**: Hold the dropper vertically and transfer 2 drops of whole blood (~50µL) to the specimen well(S) of the test device, then add 2 drops of buffer (~80 µL) and start the timer. See illustration below.
4. **For Fingerstick Whole Blood Specimen**:
   - To use capillary tube: Fill the capillary tube and transfer ~50 µL of fingerstick whole blood specimen to the specimen well(S) of the test device, then add 2 drops of buffer ~80 µL and start the timer. See illustration below.
   - To use hanging drops: Allow 2 hanging drops of fingerstick whole blood specimen (~50 µL) to fall into the center of the specimen well(S) on the test device, then add 2 drops of buffer (~80 µL) and start the timer. See illustration below.
5. Wait for the colored line is appeared. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.



INTERPRETATION OF RESULTS

POSITIVE: * Two distinct red colored lines appear. One color line should be in the control region (C) and another color 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 HCV antibodies present in the specimen. Therefore, any shade of red in the test region should be considered positive.

NEGATIVE: One red color line appears in the control region (C). No apparent red 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

Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal valid 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 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 HCV.
- 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.
- 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 HCV test cannot detect extremely low concentrations of HCV 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 C infection.
- This test will indicate only the presence or absence of HCV 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 HCV Rapid Test was based on testing of a panel of 402 HCV positive samples, 30 seroconversion panels.

In details:

- 402 HCV Antibody Positive Samples including genotypes 1, 2, 3, 4, 5 ,6. All positive samples except 1 sample are detected as positive with HCV Rapid Test.

Actual Status	CHIL HCV Rapid Test Results		
	Positive	Negative	Total
Negative	0	0	0
Positive	401	1	402
Total	401	1	402

A final sensitivity has been found 99.75%.

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

SPECIFICITY

The specificity evaluated on below samples:

- 1052 Unselected blood donors includind first time donors

From the 1052 unselected blood donors, 11 samples were positive and tested in duplicate. These resulted in 9 samples repeatedly positive and the rest 1041 samples were negative both by reference assay and CHIL HCV Rapid Test which results are demonstrated below:

Actual Status	CHIL HCV Rapid Test Results		Total
	Positive	Negative	
Negative	2	1041	1043
Positive	9	0	9
Total	11	1041	1052

A final specificity has been found 99.81%.

- 201 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 HBsAg 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 except 2 blood donors are tested as negative with HCV 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 both 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 3 negative and 3 positive samples by 20 times.

%Agreement is found as %100 for Negative samples.

%Agreement is found as %98,33 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

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3. Barrera JM, Franscis B, Ercilla G, Nelles M, Archod D, Darner J, et al. Improved detection of Anti-HCV in Post transfusion Hepatitis by third generation ELISA. Vox Sang. 1995;68:15–8

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Colin C, Lanoir D, Touzet S, Meyoud-Kraemer L, Bailley F, Trepo C HEPATITIS Group. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: An analysis of the literature. J Viral Hepat. 2001;8:87–95

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				

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