RAPINDO™
HCV
Rapid immunochromatography test for HCV Human serum/plasma

DEVICE

INTRODUCTION

RAPINDO™ HCV is a rapid, third generation two-site sandwich immunoassay for the detection of total antibodies specific to Hepatitis C virus (HCV in human serum / plasma). The test employs a genotype cross-reactive multi-epitope recombinant antigen derived from the Core, NS3, NS4 and NS5 regions of multiple HCV genotypes. The double antigen sandwich system ensures detection of all anti-HCV antibody isotopes (viz. IgG, IgM, IgA etc.) to all major HCV genotypes.

SUMMARY

HCV is a single stranded RNA virus containing a linear genome with a length of about 9,600 nucleotides with positive polarity. It is now recognised that HCV infection is the major etiological agent of post transfusion hepatitis type non-A, non-B. HCV infection frequently progresses to chronic liver disease. On the basis of phylogenetic analysis, HCV has been grouped into six major genotypes, each of which contains one or more subtypes. The distribution of HCV genotypes varies in different geographical areas.

RAPINDO™ HCV is a 3rd generation assay that uses a multi-epitope recombinant antigen that is broadly cross-reactive to all major HCV genotypes. Moreover, **RAPINDO™ HCV** detects total anti-HCV antibodies ensuring detection of all antibody isotypes viz. IgM, IgG, IgA etc.

PRINCIPLE

RAPINDO™ HCV utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components - a multi-epitope HCV recombinant antigen conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the membrane test assembly the HCV recombinant antigen-colloidal gold conjugate complexes with the anti-HCV antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit globulin - colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another multi-epitope HCV recombinant antigen coated on the membrane leading to formation of a pink to pink-purple coloured band. The absence of this coloured band in the test region indicates a negative test result.

The unreacted conjugate and unbound complex, if any, along with rabbit globulin - gold conjugate moves further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region ©, forming a pink to pink-purple coloured band. This control band acts as a procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

RAPINDO™ HCV kit has following components:

A. Individual pouches each containing:
   1. DEVICE : Membrane test assembly: Stripped with multi-epitope HCV recombinant antigen and Agglutinating sera for rabbit globulin along with HCV specific antigens and rabbit globulin - gold conjugate. Each membrane test assembly is individually pouched.
   2. Desiccant pouch.

B. BUF : Sample running buffer in a dropper bottle.

C. Package insert.

STORAGE AND STABILITY

RAPINDO™ HCV is stable to the expiry date mentioned on the label when stored at 4-30°C. Once the pouch is opened, the device must be used immediately. The shelf life of the sealed product can be seen on the packaging pouch & carton. Once the sample running buffer opened, remains stable until the expiry date stated on the buffer label. Store the Sample running buffer solution at a temperature between 4°C and 30°C.DO NOT FREEZE.

MATERIALS REQUIRED BUT NOT PROVIDED

(1) Disinfectant
(2) Disposable gloves
(3) Biohazard waste container
(4) Micropipette

SAMPLE COLLECTION

1. **RAPINDO™ HCV** uses human serum / plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. Preferably use fresh sample. However, specimen may be stored refrigerated (2-8°C) for short duration. For long storage, freeze at -20°C or below.
4. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
5. Repeated freezing and thawing of the specimen should be avoided.
6. Do not heat inactivate before use.
7. Do not use turbid, lipaemic and hemolyzed serum / plasma.
8. Do not use hemolyzed, clotted or contaminated specimens.
9. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
10. Refrigerated specimens must be brought to room temperature prior to testing.

PRECAUTIONS

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
2. Bring all reagents and specimen to room temperature before use.
3. Do not use beyond expiry date.
4. Read the instructions carefully before performing the test.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink or smoke in the area where testing is done.
9. Use protective clothing and wear gloves when handling samples.
10. Use absorbent sheet to cover the working area.
11. Immediately clean up any spills with sodium hypochlorite.
12. Dispose-off all the reagents and material used as if they contain infectious agent.
13. Do not mix components of one lot with another.
14. If desiccant colour at the point of opening the pouch has turned from blue to white, another test assembly must be run.

TEST PROCEDURE

1. Bring the sealed aluminum foil pouch of **RAPINDO™ HCV** membrane test assembly to room temperature.
2. Open a foil pouch by tearing along the "notch".
3. Remove the membrane test assembly. Once opened, the membrane test assembly must be used immediately.
4. Label the membrane test assembly with specimen identity.
5. Place the membrane test assembly on a flat horizontal surface.
6. Carefully dispense 10 μl of serum / plasma into the specimen port "A".
7. Add **three drops** of sample running buffer into port "B".
8. Observe the development of visible colored band at Test region.
9. Positive result may be observed within 15-20 minutes. Do not read results after 20 minutes.
10. The test should be considered invalid if the control band "C" does not appear. The test is also invalid if neither the control nor the test bands appear. Repeat the test with a new **RAPINDO™ HCV** membrane test assembly.

INTERPRETATION OF RESULTS

NEGATIVE:
If antibodies to HCV are not present, only one colored band at Control window (C) would appear.

POSITIVE:
If antibodies to HCV are present, two colored bands appear at Test (T) and Control (C) windows.

INVALID:
The test should be considered invalid if neither the test nor the control bands appear. Repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

In an external evaluation, the performance of **RAPINDO™ HCV** was evaluated using a panel of fifty known positives (of varying reactivity) and one hundred and fifty known negative specimens in comparison to a Panel plasma (ECLIA). The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	RAPINDO™ HCV	ECLIA
Number of specimens tested	569	200	200
Number of Positives	50	50	50
Number of Negatives	150	150	150

Based on this evaluation:
Sensitivity of **RAPINDO™ HCV**: 100% and Specificity of **RAPINDO™ HCV**:100%

LIMITATIONS OF THE ASSAY

1. Approximately 25-30% of individuals with chronic HCV infections have persistently normal alanine aminotransferase (ALT or SGPT) level and these individuals are usually referred to as 'healthy carrier' of HCV. However, several studies have demonstrated that the histological features of most healthy carriers showed chronic liver damage of a variable degree, ranging from mild hepatitis to liver cirrhosis, and thus the existence of the true 'healthy carrier' of HCV is still debatable.
2. At least six major genotypes of HCV, each comprising multiple subtypes, have been identified worldwide. Apart from genotypes 1 to 6, HCV genotypes 7, 8 and 9 have been identified only in Vietnamese patients, and genotypes 10 and 11 were identified in patients from Indonesia. There has been disagreement about the number of genotypes into which HCV isolates should be classified. Investigators have proposed that genotypes 7 through 11 should be regarded as variants of the same group and classified as a single genotype, type 6.
3. **RAPINDO™ HCV** detects total antibodies that include IgG, IgM, IgA etc. although it has been reported that IgM response in HCV infection is variable, its simultaneous detection along with IgG and other isotypes appear to be advantageous in comparison to IgG-only detection assays. These is because some studies indicate IgM anti-HCV as the first marker for active antibody response and seroconversions particularly in post transfusion non-A non-B hepatitis & liver transplant patients. However, other studies show that IgM anti-HCV is not always limited to acute phase of the disease, since long-term chronic patients had protracted periods of IgM anti-IgM reactivity. The performance of **RAPINDO™ HCV** is not affected by this variability because it also detects IgG simultaneously which is present in all stages of infection.
4. Though **RAPINDO™ HCV** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HCV infection.
5. Absence of antibodies to HCV does not indicate that an individual is absolutely free of HCV infection as the collection of sample and its timing vis-à-vis seroconversion will influence the test outcome.
6. Do not compare the intensity of the test lines and control lines to judge the concentration of antibodies in the test specimen.
7. Since various test of HCV differ in their performance characteristics and antigenic composition, their reactivity pattern may differ.
8. Testing of pooled samples is not recommended.
9. The membrane is laminated with an adhesive tape to prevent the surface evaporation. Air pockets or patches may appear, which do not interfere with the test results. Presence of a band at test region even if low in intensity or formation is a positive result.
10. The deliberate slow reaction kinetics of **RAPINDO™ HCV** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.
11. Most positive results develop within 15 mins. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only after 20 minutes. Do not read results after 20 minutes.
12. As with all the diagnostics tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
13. **RAPINDO™ HCV** should only be used as a screening tests and its result should be confirmed by other supplemental method before taking clinical decisions.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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SYMBOL KEYS

Temperature Limitation	Consult Instruction for Use	Manufature Date
Professional use only	IVD — In-vitro Diagnostics Medical Device	This way up
Use by	REF — Catalogue Number	DEVICE — Device
Contains Sufficient for <n> testes	LOT — Batch No. / LOT No.	
Do Not Reuse	BUF — Sample Running Buffer	PIPETTE — Sample Applicator

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