Size : 137 x 218 mm

Flavicheck®-HCV WB

Rapid test for the detection of total antibodies to HCV in human serum / plasma / whole blood

DEVICE

INTRODUCTION

Flavicheck®-HCV WB is a rapid, fourth generation two site sandwich immunoassay for the detection of total antibodies specific to Hepatitis C virus (HCV in human serum / plasma / whole blood). The test employs a genotype cross-reactive recombinant peptide 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 isotypes (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 recognized 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.

The first-generation HCV antibody test became commercially available in early 1990s and was widely used. As more reactive recombinant antigens were identified from conserved regions of the HCV genome, newer serologic assays (second and third generation) were introduced.

Third-generation assays were introduced in the late 1990s. In these assays, a recombinant NS5 antigen has been added to the antigens used in the second-generation assays. These third-generation assays have higher sensitivities and specificities than second-generation assays and are much less strongly influenced by the infecting genotype.

The first, second and third generation HCV antibody assays still lack sensitivity in seroconversions or show inexplicable discrepancies with confirmatory assays. This is primarily due to poor cross-reactivity with the current HCV genotype 1 antigen-based assays. To solve this problem Fourth Generation assays using antigens from multiple HCV genotypes that includes genotypes 2 & 3 apart from genotype 1, containing universally conserved epitopes, are been developed and evaluated.

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

PRINCIPLE

Flavicheck®-HCV WB 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 Peptide 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 Peptide 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 Peptide 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 move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region (C), 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

Each kit contains:

A. Individual pouches each containing:
   1. DEVICE : Membrane test assembly pre-dispensed with multi-epitope HCV Recombinant Peptide Antigen-colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, multi-epitope HCV Recombinant Peptide Antigen and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. Desiccant pouch.
   3. PIPETTE : Disposable plastic sample applicator.

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

C. Package insert.

REF	402060001	402060010	402060025	402060050	402060100
Σ	1 Test	10 Tests	25 Tests	50 Tests	100 Tests

STORAGE AND STABILITY

The sealed pouches in the test kit and the kit components may be stored between 4-30°C for the duration of the shelf life as indicated on the pouch / carton label. DO NOT FREEZE.

NOTE

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use.
2. Do not use beyond expiration date.
3. Read the instructions carefully before performing the test.
4. 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.
5. Handle all specimens as if potentially infectious.
6. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
7. If desiccant colour at the point of opening the pouch has turned from blue to pink, another test device must be run.

SPECIMEN COLLECTION AND PREPARATION

1. **Flavicheck®-HCV WB** uses human serum / plasma / whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. For whole blood, collect blood with a suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Do not freeze whole blood specimens.
5. Though fresh specimen is preferable, in case of delay in testing, it may be stored at 2-8°C for maximum up to 24hrs.
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Do not use turbid, lipaemic, hemolysed, and clotted or contaminated specimen.
8. Repeated freezing and thawing of the specimen should be avoided.
9. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
10. Do not heat inactivate the specimen.
11. Refrigerated specimens must be brought to room temperature prior to testing.

TEST PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the sealed aluminium foil pouch of **Flavicheck®-HCV WB** device to room temperature.
2. Open a foil pouch by tearing along the "notch".
3. Remove the testing device and the sample applicator. Once opened, the device must be used immediately.
4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Holding the sample applicator vertically, carefully dispense exactly two drops (50 µl) of serum / plasma / whole blood into the specimen port "A".
7. Add three drops of sample running buffer into the port "B".
8. At the end of 15 minutes read results as follows:

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

Positive
If antibodies to HCV are present, two coloured 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

Internal Evaluation-I

In an in-house study, the performance of **Flavicheck®-HCV WB** device was evaluated using a panel of fifty known positives (of varying reactivity) and five hundred and nineteen known negative specimens in comparison to a licensed HCV-ELISA kit. The results of the evaluation are as follows:

Specimen Data	Total	Flavicheck®-HCV WB	Licensed ELISA
Number of specimens tested	569	569	569
Number of Positives	50	50	50
Number of Negatives	519	517	518

Based on this evaluation:
Sensitivity of **Flavicheck®-HCV WB** : 100%.
Specificity of **Flavicheck®-HCV WB** : 99.61%.

Internal Evaluation-II (Precision study)

Intra-assay Precision study

One PCR-positive sample was assayed 10 times on the same day.

Results: No variation in results was observed indicating 100% correlation.

Inter-assay Precision study

One PCR-positive sample was assayed 3 times on 3 different days.

Results: No variation in results was observed indicating 100% correlation.

Independent External Evaluation-I

To check the specificity of **Flavicheck®-HCV WB**, one thousand and three PCR-confirmed negative samples were tested with **Flavicheck®-HCV WB** by a CE-accredited reputed reference laboratory in Europe. The results of the evaluation are as follows:

Total number of PCR-confirmed negative samples tested	1003
Number of specimens that showed negative with **Flavicheck®-HCV WB**	1001
Number of specimens that showed positive with **Flavicheck®-HCV WB**	2

Based on this evaluation:

Specificity of **Flavicheck®-HCV WB** : 99.80 %.

Independent External Evaluation-II

In another independent external study performed by a CE-accredited reputed reference laboratory in Europe, the specificity of **Flavicheck®-HCV WB** was evaluated with a panel of hundred potentially cross-reacting sera samples. This included positive samples of HBsAg, HTLV, CMV, VZV, EBV, HSV, HAV and HIV 1&2. The results of the evaluation are as follows:

Total number of samples tested	100
Number of specimens that showed negative with **Flavicheck®-HCV WB**	100
Number of specimens that showed positive with **Flavicheck®-HCV WB**	0

Based on this evaluation:

Specificity of **Flavicheck®-HCV WB** : 100 %.

REMARKS

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 through 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. **Flavicheck®-HCV WB** detects total HCV 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 & other isotypes appears to be advantageous in comparison to IgG-only detection assays. This is because some studies indicate IgM anti-HCV as the first marker for active antibody response and seroconversion 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 the acute phase of the disease, since some long-term chronic patients had protracted periods of IgM anti-HCV reactivity. The performance of **Flavicheck®-HCV WB** is not affected by this variability because it also detects IgG simultaneously which is present in all stages of infection.
4. **Flavicheck®-HCV WB** uses a novel recombinant peptide antigen (RPA). Traditionally, recombinant antigens are prepared by "cutting" the target gene of the pathogen by restriction digestion and "joining" it with the plasmid of the vector (like *E. coli*) by ligase. Subsequently the vector is grown, lysed and purified recombinant antigen is extracted. RPAs, also known, as fusion proteins are recombinant antigens but prepared in a slightly different method. The amino acid residues of conserved immunodominant epitopes are identified. An oligoneucleotide corresponding to the amino acid sequence of the epitope is synthesized. This oligoneucleotide is incorporated into the plasmid of the vector to produce the RPA or fusion protein. RPAs, being short chain molecules, confer high sensitivity & specificity when compared to traditional recombinant antigens. **Flavicheck®-HCV WB** uses a novel multi-epitope RPA that is broadly cross-reactive to all major HCV genotypes.
5. Whole blood samples, when used directly from finger-prick (without anticoagulant,) should be free from fibrin and microclots to avoid altered flow properties and delayed reaction time.
6. Though **Flavicheck®-HCV WB** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HCV infection.
7. 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.
8. Do not compare the intensity of test lines and the control lines to judge the concentration of antibodies in the test specimen.

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9. Since various tests for HCV differ in their performance characteristics and antigenic composition, their reactivity patterns may differ.
10. Testing of pooled samples is not recommended.
11. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pockets or patches may appear, which do not interfere with the test results. Presence of a band at the test region even if low in intensity or formation is a positive result.
12. The deliberate slow reaction kinetics of **Flavicheck®-HCV WB** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.
13. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only after 30 minutes. Do not read results after 30 minutes.
14. As with all diagnostic 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.
15. **Flavicheck®-HCV WB** should only be used as a screening test and its results 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 Instructions for use	Date of Manufacture	DEVICE — Device
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	BUF — Wash Buffer
Use by	REF — Catalogue Number	Do not reuse	PIPETTE — Disposable Plastic Sample Applicator
Σ — Contains sufficient for <n> tests	LOT — Batch Number / Lot Number		

Manufactured by:

Qualpro Diagnostics

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