VANSCAN™

HCV
Microwell ELISA

Enzyme immunoassay for the detection of Antibodies to Hepatitis C Virus in Human Serum or Plasma

INTENDED USE:

HCV Microwell ELISA is an *in vitro* enzyme immunoassay for the qualitative determination of Antibodies to Hepatitis C Virus in Human Serum or Plasma.

INTRODUCTION:

Viral Hepatitis, is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis, seen in children and adults, are caused by Hepatitis A virus, Hepatitis B Virus or Hepatitis C Virus.

Hepatitis C Virus was identified as the major causative agent of non-A, non-B Hepatitis (NANBH) and liver disease. Studies throughout the world indicate that the virus is transmitted through sexual contact, contaminated blood and blood products, blood transfusion and other close personal contacts. HCV is an evolved virus having a single positive stranded RNA genome.

PRINCIPLE OF THE TEST:

The HCV Microwell ELISA employs an immunosorbent of recombinant antigens, core, NS3, NS4 and NS5 bound to the wells of the microplate.

During the course of the assay, diluted controls and specimens are added to the wells and incubated HCV Antibodies, if present, will bind to the Immunosorbent.

After a thorough washing of the wells to remove unbound antibodies and other serum components, a standardised preparation of Horse-Radish Peroxidase - conjugated to goat antibodies specific for Human IgG is added to each well. After a second incubation to allow the antibodies to react and washing to remove any unbound HRP-conjugated Antibodies, Substrate Solution containing Tetramethylbenzidine (TMB) and hydrogen peroxide is added to each well . Colour develops in proportion to the amount of HCV specific antibodies present, if any, in the specimen tested. The Enzyme–Substrate reaction is terminated by the addition of acid to each well.

The absorbance is read at 450 nm (reference wavelength 630nm) within 15 minutes on a standard microplate Reader.

KIT CONTENTS:

1. **HCV Antigen Coated Microplate/Microstrips**

   One plate of 96 microwells coated with HCV antigens. If less than the whole plate is being used, allow the wells to each room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C. Once opened microwells should be used within one month.

2. **Sample Diluent**

   One bottle containing buffered solution containing proteins, stabilizer, preservative and indicator dye for sample addition.

3. **Negative Control**

   One vial containing 0.3 ml of normal human serum with preservative. Negative Control has been tested and found to be negative for anti-HIV 1+2, HBsAg, Anti-HCV and Syphilis.

4. **Positive Control**

   One vial containing 0.3 ml of inactivated human serum in a buffer containing protein with preservative. Positive Control has been tested and found to be negative for HBsAg, Anti-HIV 1+2 and Syphilis.

5. **Conjugate Concentrate (101X)**

   One vial containing Anti-human IgG conjugated to HRP with protein stabilizers and preservatives. Bottle containing 101 times working strength antibody conjugates. For preparation of Working Conjugate Solution refer to Step wise Procedure Card provided along with this Kit.

6. **Conjugate Diluent**

   One bottle containing solution consisting of buffer and preservatives. For preparation of Working Conjugate Solution refer to Step wise Procedure Card provided along with this Kit.

7. **TMB Substrate (101x):**

   One vial containing 0.3ml of 3,3',5',5'- Tetramethyl benzidine - TMB, (101x) containing Dimethyl Sulfoxide (DMSO) as solvent.

8. **Substrate Buffer:**

   One bottle containing 15ml of Substrate Buffer containing Hydrogen Peroxide (H_2O_2) and Stabilizers. For Preparation of Working TMB Substrate solution, refer to the preparation of Working Reagents.

9. **Wash Solution Concentrate (20X)**

   One bottle containing 20 times working strength phosphate buffer saline Wash Solution with detergent. For preparation of Working Wash Solution, refer to Step wise Procedure Card provided along with this Kit.

10. **Stop Solution**

    One bottle containing colourless solution of diluted acid and stabilizers.

11. **Microtrip Covers**

    Sheets to seal microstrips during incubation.

12. **Package Insert**

13. **Procedure Card**

MATERIALS REQUIRED BUT NOT PROVIDED:

(A) Distilled or deionized water, (B) Graduated Cylinder for reagents dilutions, (C) Micropipettes, (D) Paper towels or absorbent paper, (E)Timer, (F) Incubator, maintained at 37±1°C, (G) ELISA Reader, (H) ELISA Washer, (I) Sodium hypochlorite solution (free available chlorine 50-500mg/dl) and (J) Disposable gloves.

SPECIMEN TRANSPORT AND STORAGE

Store the samples at 2-8°C. Samples not required for assay within 7 days should be stored frozen (-15 °C or colder). Avoid multiple freeze-thaw cycles. After thawing ensure samples are thoroughly mixed before testing.

PRECAUTIONS

1. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% Sodium Hypochlorite before work is continued. Sodium Hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed off as potentially Bio-Hazardous waste. Do not autoclave materials containing's Sodium Hypochlorite.
2. Do not pipette by mouth. Wear disposable gloves and eye protection while handlings specimens and performing the assay. Wash hands thoroughly thereafter.
3. Sulphuric acid used in Stop Solution is corrosive and should be

handled with appropriate care. If it comes into contact with skin or eyes, wash thoroughly with water. If any of the reagents come into contacts with skin or eyes wash the area extensively with water.

4. Do not use the reagents beyond the stated expiry date.
5. Do not modify the test procedure or substitute reagents from other manufactures or other lots unless the reagents are stipulated as interchangeable.
6. Do not reduce any of the recommended incubation times.
7. Allow the reagents and samples to come to 18 to 30°C before use. Immediately after use, return reagents to the recommended storage temperature.
8. Do not allow wells to become dry during the assay procedure.
9. Do not cross-contaminate reagents. It is recommended to use dedicated separate pipettes for dispensing the Substrate Solution and Conjugate.
10. Ensure that the bottom of the plate is clean and dry and that no bubbles are presents on the surface of the liquid before reading the plate.

PREPARATION OF TEST:

1. Bring all the reagents to room temperature 30 minutes before use.
2. Take the required number of strips from the sealed, antigen coated plate. The remaining strips must be kept at 2-8°C with the silica gel after proper sealing of the pouch.

TEST PROCEDURE

1. Take the required number of strips and fix them on to the plate.
2. Pipette 100µl of Sample Diluent into each plate well except Blank well A1 and pipette 10 µl of Negative Control into each well from B1 to D1 and 10 µl of Positive Control into each well from E1 to F1, respectively, and then pipette 10 µl of each sample into the remaining wells.
3. Incubate at 37±1°C for 30 minutes after sealing the plate with the Microstrip Covers.
4. Before the last 5-10 minutes of the 1st incubation, make a 1:100 dilution of the Conjugate with Conjugate Diluent. Refer to the Step Wise Procedure Card provided along with this kit.
5. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution. Refer to the Step Wise Procedure Card provided along with this kit.
6. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100µl of prepared Working Conjugate Solution into each well except Blank well A1.
7. Incubate the plate at 37±1°C for 30 minutes after sealing it with the Microstrip Covers.
8. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution.
9. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100 µl of working Substrate Solution into each well including the Blank well A1. Refer to the Step Wise Procedure Card provided along with this kit.
10. Incubate it at controlled room temperature (21-25°C) for 30 minutes.
11. Pipette 50µl of Stop Solution into each well including Blank well A1.
12. Read the absorbance at 450nm (reference wavelength at 630nm) within 15 minutes after pipetting the Stop Solution.

QUALITY CONTROL

Results of an assay are valid if the following criteria for the Controls are met:-

(a) **Absorbance of Blank** should be less than **0.050**

(b) **Mean absorbance of Positive Controls** should be **> 1.000**

(c) **Mean absorbance of Negative Controls** should be **< 0.100**. If absorbance of Negative Control comes in negative. eg.- 0.003 it should be considered as zero.

If the results are outside the above ranges, then the test should be conducted again.

CUT OFF VALUE:

Calculation of the Cut off Value (COV).

(a) Calculate the Negative Control mean ($NC\bar{x}$)

e.g. Negative Control 1 absorbance = 0.009
Negative Control 2 absorbance = 0.010
Negative Control 3 absorbance = 0.011
Negative Control Mean ($NC\bar{x}$) =
(0.009+0.010+0.011)/3 = 0.010

(b) Calculate the Cut off Value (COV)

Cut off Value (COV) = $NC\bar{x}$ +0.200=0.010+0.200=0.210

INTERPRETATION OF RESULTS

Reactive Results:- Samples with absorbances greater than or equal to the Cut off Value are considered Reactive for HCV.

Non-reactive Results:- Samples with absorbances less than the Cut off Value are considered non-reactive for HCV.

NOTE:- If the samples are considered reactive, the test should be conducted two more times.

Gray zone Samples : whose absorbance falls in between ± 10% of Cut-off-Value (COV).

In cases where the re-tests show non-reactive, the samples are considered non-reactive, and on the other hand, if one of the re-tests shows reactive, the samples are considered reactive.

The samples considered reactive should be tested again by Western Blot and RIBA etc. for final interpretation.

PERFORMANCE CHARACTERISTICS

Sensitivity	100%
Specificity	99.5%

BIBLIOGRAPHY:

1. Tang, E.,(1991) Hepatitis C virus. A review. West Med.;155(2):164-168
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3. Vrielink, H.,Reesink, H.W., et al. (1997) Performance of three generations of anti-hepatitis C virus enzyme-linked immunosorbent assays in donors and patients . Transfusion; 37(8):845-849.

VANGUARD DIAGNOSTICS

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