ImmunoScience INDIA

ImmunoELISA HCV

"ELISA Test for Detection of Antibodies to HCV"

Store at 2 °C to 8 °C

INTENDED USE:

ImmunoELISA HCV is an enzyme-linked immunosorbent assay for the qualitative detection of antibody to hepatitis C virus (anti-HCV) in human serum and plasma. It is intended for professional use only as an aid in the identification & diagnosis of infection with Hepatitis-C virus.

It is also intended for use as a donor screening test to detect antibodies to hepatitis C virus in plasma and serum samples from individual human donors, including volunteer donors of Whole Blood, blood components, source plasma, and other living donors.

Any reactive specimen with the **ImmunoELISA** HCV Kit must be confirmed with alternative testing method(s) and correlate with clinical findings.

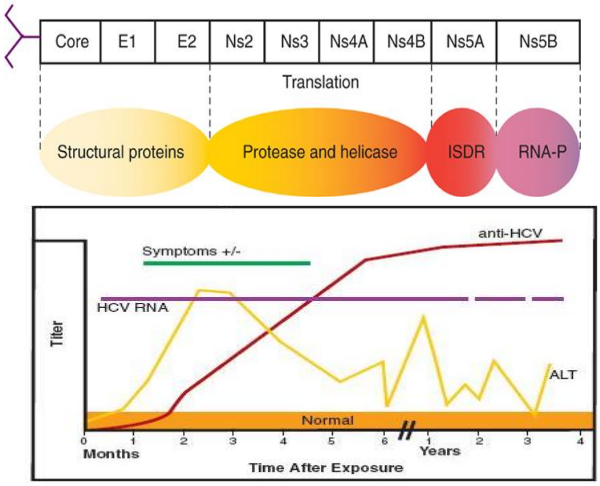
SUMMARY OF TEST:

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 although 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. The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Second generation tests, which included recombinant/synthetic antigens from the Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. Clinical studies show that significant amount of HCV infected individuals develop antibodies to NS5 non-structural 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.

At least 80% of infected persons will develop chronic disease. Cirrhosis occurs in 20- 50% of chronic HCV patients after a period of 20 years and the annual incidence of hepatocellular carcinoma in cirrhotic is approximately 3%. Detection of specific antibody against particular antigens of HCV is critical for the diagnosis of infected patients. Among various methods which are exploited for the detection of HCV antibody.

ELISA test offers reliability and high sensitivity particularly in preliminary and screening diagnosis. This kit has third generation HCV ELISA format with high sensitivity and specificity and detects specific antibody against HCV

Hepatitis C Genome



PRINCIPLE OF THE TEST:

ImmunoELISA HCV test principle is based on indirect enzyme immunoassay. The Micro titer wells are coated with cocktail of HCV recombinant antigens including NS3,NS4,NS5 and CORE antigens. Then serum samples are allowed to react with solid phase antigens. If HCV-specific antibodies are presented in the human specimen they will bind to HCV antigens through their individual antibody Fab' section. After incubation, the wells are washed to remove unbound antibodies and anti-human antibodies IgG antibody conjugated with HRP is added into the wells following another incubation and wash step. A solution of TMB is added and incubated at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of specific anti–HCV is directly proportional to the color intensity of the test sample.

KIT COMPONENTS (1 X 96 TEST)

1. **Microwell plate (1x96 wells)**: Each well coated cocktail of HCV recombinant antigens (Core+NS3+NS4+NS5) in bicarbonate buffer (solvent). The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately.
2. **Specimen/Sample Diluent (1x 20 ml):** One bottle contains Phosphate buffer saline, Triton-X -100 (as surfactant), Bovine serum albumin (protein stabilizer) and Proclin-300 (as preservative) 0.1% v/v.
3. **Negative Control (1x 0.5 ml):** One bottle contains normal human serum and Proclin-300, 0.1% v/v as preservative.
4. **Positive Control (1x 0.5 ml):** One bottle contains inactivated anti HCV human serum and Proclin-300, 0.1% v/v as preservative.
5. **HCV specific HRP Conjugate concentrate, 51x (1x 0.6 ml)**: one bottle contains recombinant HCV antigen/peroxidase conjugate, Bovine serum albumin (as stabilizer) and gentamycin sulphate 0.005% and Proclin-300, 0.05% v/v (as preservative).
6. **Conjugate Diluent (1x 20 ml)**: one bottle contains phosphate saline-casein buffer and Proclin-300 0.05% v/v (as preservative).
7. **TMB Substrate, 101x concentrated (1x0.3 ml)**: one bottle contains tetramethyl benzidine in Dimethyl sulphoxide as solvent.
8. **Substrate Buffer (1x20ml)**: one bottle contains Citrate-Acetate buffer (solvent) containing hydrogen peroxide 0.006% v/v.
9. **Wash Buffer concentrate 20x (1x50ml)**: one bottle contains concentrated phosphate buffered saline with polysorbate (surfactant) and Proclin-300 (preservative), 0.05 %v/v.
10. **Stop Solution (1x20ml)**: one bottle contains 1.5 N sulphuric acid.
11. **Instruction manual/product Insert.**

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Micro pipette capable of delivering 10 µl, 50 µl, 75 µl, and 100 µl volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells
4. Parafilm or other adhesive film sealant for sealing plate
5. Timer
6. Distilled or de-ionized water.

SPECIMEN COLLECTION AND REPARATION:

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with serum or plasma specimen without additives only.

3. If a specimen is not tested immediately, the serum/plasma shall be separated and refrigerated at 2°C - 8°C. If storage period greater than three days are anticipated, the separated serum/plasma should be frozen (-20°C).
4. Avoid repeated freezing-thawing of specimens.
5. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
6. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
7. Do not use serum specimens demonstrating gross lipemic or lipemia, gross hemolysis or turbidity.
8. Do not use specimens containing sodium azide as preservative.

REAGENT PREPARATION

A. Wash Buffer preparation: Dilute 20x wash buffer provided in the kit in 1:20 ratio in purified water. For example, to prepare 1000 ml wash buffer, mix 50 ml of 20x wash buffer into 950 ml of purified water. Decide the volume of buffer according to the required number of test and dead volume of washer instrument. In case of manual washing, the volume can be calculated accordingly.

B. HRP Conjugate preparation: Dilute HRP conjugate concentrate (51x) in conjugate Diluent (1:51 ratio) as follows:

Strips	1	2	3	4	5	6	7	8	9	10	11	12
HRP C. 51x (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conj. Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

C. TMB substrate preparation: Dilute TMB substrate concentrate (101x) in Substrate Buffer (1:101 ratio) as follows:

Strips	1	2	3	4	5	6	7	8	9	10	11	12
TMB S. 101x (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Substrate Buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

- ✓ Prepare the reagents just before use.
- ✓ Prepare only the required quantity of reagents.
- ✓ Ensure the TMB substrate to be kept in low light and closed container.
- ✓ Discard the remaining reagent after use.

ASSAY PROCEDURE

1. Take the required number of strips and fix them to plate.
2. Pipette 100 µl of sample diluent into each plate well and pipette 10 µl of Negative control into each well from 1A to 1C and 10 µl of Positive Control into each well from 1D to 1E, respectively and then, pipette 10 µl of each specimen into the remaining well.
3. Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
4. Seal the microwell strips using the plate cover and incubate at 37°C ± 1°C for 30 minutes.
5. Before 5 to 10 minutes of completion of the 1st incubation, make 1:51 dilution of HRP conjugate with conjugate diluents.
6. Aspirate the contents from all the wells and wash 5 times with 300 to 350 µl of diluted washing solution. (325 µl/well/time)
7. Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 µl of prepared diluted HRP conjugate into each well.
8. Incubate at 37°C ± 1°C for 30 minutes after sealing the plate with cover.
9. Before 5 to 10 minutes of 2nd incubation, make 1:101 dilution of TMB substrate with substrate buffer.
10. Aspirate the contents from all the wells and wash 5 times with 300 to 350 µl of diluted washing solution. (325 µl/well/time)
11. Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 µl of prepared diluted TMB substrate into each well. And incubate at controlled room temperature (21°C - 25°C) in dark environment for 15 minutes.
12. Pipette 100 µl of stop solution into each well and tap the plate gently to homogenize the coloring materials.
13. Read the absorbance at 450 nm (reference wavelength at 630 nm) against an air blank within 30 minutes after pipetting of stop solution.

QUALITY CONTROL

1. The average absorbance of Positive Control (PCx) should be greater than or equal to 1.0.
2. The average absorbance of Negative Control (NCx) should be less than or equal to 0.200.

INTERPRETATION OF RESULTS

(a) Calculate the negative control mean (NCx)
Ex. Negative Control 1 absorbance=0.045
Negative Control 2 absorbance=0.050
Negative Control 3 absorbance=0.055
Negative Control Mean (NCx)=
(0.045+0.050+0.055)/3=0.050

(b) Calculate the positive control mean (PCx)
Ex. Positive Control 1 absorbance=1.841
Positive Control 2 absorbance=1.872
Positive Control Mean (PCx)= (1.841+1.872)/2=1.8565

(c) Calculate the cut off value
Cut off value= NCx + 0.200

INTERPRETATION

Samples with absorbance greater than or equal to the Cut Off value are considered positive to anti-HCV. Samples with absorbance less than Cut Off value are considered negative to Anti-HCV. Sample values within the ±10% of cut off value should be considered as indeterminate or grey zone samples. It should be retested again or repeat with freshly collected sample.

WARNING AND PRECAUTIONS:

1. This product is made for in Vitro Diagnostic Use only.
2. Package insert must be read completely before performing the test. Failure to follow the instructions in pack insert may give inaccurate test results.
3. Do not use the Kit beyond the expiry date.
4. Bring all reagents& samples to room temperature (18°C-28°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. In the beginning of each incubation and after adding Stop Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as it may give inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells.
11. Don't allow the micro plate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
13. The TMB substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16. Avoid strong light or sunlight during color development.

LIMITATIONS:

1. The **ImmunoELISA HCV**is intended to detect antibodies against HCV. However, the test cannot detect the quantity or relative change in the levels of antibodies.
2. The product is not made for detection of HCV Antigen.
3. The product is designed so as to give best results but, some samples may show cross reactivity. Hence, there may be a chance of false positive results. Every positive result shall be verified by testing with alternate kit or methods and also shall be correlated with symptoms.

REFERENCES:

1. Choo, Q.L., G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, and M. Houghton. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244:359
2. Kuo, G., Q.L. Choo, H.J. Alter, and M. Houghton. An assay for circulating antibodies to a major etiologic Virus of human non-A, non-B hepatitis. Science 1989; 244:362
3. van der Poel, C. L., H.T.M. Cuypers, H.W. Reesink, and P.N.Lelie. Confirmation of hepatitis C Virus infection by new four-antigen recombinant immunoblot assay. Lancet 1991; 337:317
4. Wilber, J.C. Development and use of laboratory tests for hepatitis C infection: a review. J. Clin. Immunoassay 1993; 16:204.

IVD	In Vitro Diagnostic Use
	Manufacturer
	Manufacturing Date
	Expiry Date
LOT	Lot Number
	Store at + 2°C to + 8°C
	Single Use
Σ	Number of tests in the pack
	Do not use if pouch or kit damaged
	This side Up
	Read package insert beforeuse

MANUFACTURED BY

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