

Anti-HCV ELISA

REF: 1308 001

96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the qualitative determination of Anti-HCV (Hepatitis C Virus Antibody) (IgG & IgM) in human serum or plasma

Background

Hepatitis C virus is a single stranded RNA virus with some structural relations to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provides the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins. HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily through human blood or body fluid are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most people in developing countries .

Assay Principle

This assay is based upon the two-steps indirect method. In the first step, sample and recombinant HCV coated microwells are combined. During the incubation, the Anti-HCV present in sample binds to the antigen coated on the wells. Unbound serum proteins are removed during the washing step. After the washing, in the second step, enzyme(Horseradish peroxidase) conjugate is added to the reaction mixture. During the incubation, the Anti-HCV captured to the solid phase in the first step reacts with human IgG and IgM within enzyme conjugate. Then a complex is generated between the solid phase, the Anti-HCV within the sample and the human IgG and IgM in the enzyme conjugate by immunological reactions. After a second washing to remove unbound conjugate, substrate(TMB solution) is added resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is proportional to the amount of Anti-HCV in the sample.

Reagents

Microplate (MP)

12 breakable 8 wells plate (Total 96 wells) coated with recombinant HCV antigens.

Negative Control

Human serum non reactive for HBsAg and antibodies to HIV-1, HIV2 and HCV.

Positive Control

Polyclonal antibodies to HCV: Core NS3,NS4, NS5 conjugated with human IgG.

Assay buffer

Buffered saline.

Enzyme Conjugate

Solution containing mouse monoclonal anti-human IgG and IgM conjugated with HRP.

Substrate (TMB solution): 3,3',5,5'-tetramethyl benzidine solution in citrate buffer containing hydrogen peroxide

Wash Solution Concentrate

Wash solution H, 20X concentrated:surfactant in buffered saline

Stop Solution

1 N HCl solution

Plastic bag with ziplock closure (for storage of unused strips)

Materials Required but not Provided

- 1- Automatic pipettes
- 2- Shaker/incubator
- 3- Equipment for rinsing wells.
- 4- Elisa reader
- 5- Vortex tube mixer.
- 6- Disinfectant
- 7- Adsorbent material

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in-vitro diagnostic use		Use by/Expiration Date
	Batch Code/Lot number		CAUTION. Consult instructions for use
	Catalogue Number		Manufactured by
	Consult instructions for use		

Reagents Storage and Stability

- 1- Avoid exposure to direct sunlight during incubations;
- 2- Kit is stored at 2-8°C and stable till the expiration date. After initial opening the kit is stable for 1 months if stored at 2-8 °C. If used in several separate experiments, kit contents should be stored as follows:
 - Unused strips: in a firmly closed ziplock bag at 2-8 °C until the expiration date;
 - Opened vials with controls , conjugate, assay buffer and substrate: at 2-8 °C for 1 month after opening;
 - Opened vials with concentrated wash solution and stop solution: at +2-8°C until the expiration date;
 - Wash solution prepared for use: at room temperature for 5 days.

Reagent Preparation

1. Bring all reagents to room temperature (18-25 °C) prior to use for at least 30 minutes.
2. Negative and Positive liquid controls are ready to use.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled water and mix well. Prepare the required volume according to the number of assayed samples.
For example: Add 5ml of wash solution to 95ml of distilled water.
- 4- Protect substrate from direct light.

Specimen Collection and Preservation

- 1- Serum and plasma are suitable for analysis. The only acceptable anticoagulants are heparin, EDTA and sodium citrate. Specimen should be promptly separated from cells after blood collection.
- 2- Cap and store the samples at 18-25 °C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 °C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 °C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. After thawing, bring to room temperature and mix well by gently shaking.

Precautions and warnings

1. Despite being confirmed as negative to all viruses, It is recommended that all human sourced controls be considered potentially infectious.
2. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
3. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
4. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
5. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
6. Do not touch or splash the rim of the well with conjugate.
7. Do not mix or use components from kits with different batch codes.
8. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.
9. It is important that the time of reaction in each well is held constant to achieve reproducible results.
10. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and Stop Solution should be added in the same sequence to eliminate any time deviation during reaction.
11. When handling conjugate vials, change gloves that have contacted human plasma/sera since introduction of human IgG/IgM will result in a neutralized conjugate.

Assay Procedure

1. Pipette 80 µL of assay buffer into each well, except well A1 (blank).
2. Pipette 40 µL of controls or samples: negative in triplicate and positive in duplicate, patients samples in duplicates or monoplicates.
3. Incubate for 30 minutes at +37 °C while shaking(500–700 rpm).
4. Wash wells 4 times
5. Pipette 100 µL of conjugate into each well,except well A1.
6. Incubate strips for 30 minutes while shaking (500– 700 rpm) at +37 °c.
7. Wash wells 4 times.
8. Pipette 100 µl of substrate into each well.
9. Incubate for 10 minutes while shaking (500–700 rpm) at +37 °C or for 20 minutes without shaking at room temperature in the dark.
10. Add 100 µL of stop solution into each well and shake for 1-2 min at room temperature.
11. Read the absorbance at 450 nm within 20 min

Assay Scheme	
Assay buffer	80 µl
Controls or sample	40 µl
Incubation time	30 minutes
Washing step	4 times
Enzyme Conjugate	100 µl
Incubation time	30 minutes
Washing step	4 times
Substrate	100 µl
Incubation time in dark	20 minutes
Stop solution	100 µl
Read absorbance	450 nm

Wash procedure

- 1–Remove the contents of the wells into container with disinfectant;
- 2–Dispense 300 µl of wash solution, , shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 4 times;
- 3–Strike the wells sharply on absorbent material to remove any liquid residue.

Data Processing

The recommended control requirement for this assay is using positive and negative controls to verify assay performance. The result is valid if the following criteria for the controls are both met

Positive Control

Mean absorbance of Positive Control is equal to or higher than 0.5

Negative Control

Mean absorbance of Negative Control is lower than 0.2

Note: The test is considered not valid if the mean absorbance of the negative control is more than 0.2 and samples must be retested.

Results

Each plate must be considered separately when calculating and interpreting results of the assay.

Cut-off Value

Calculate the mean absorbance of the replicates of the Negative Control.

The cut-off = C_{crit} + Mean of the Negative Control replicates.
 Note: C_{crit} is a specified coefficient defined by the Manufacturer.
 C_{crit} for each particular lot is specified on the kit label

Example

Mean Negative Control $(0.030 + 0.022 + 0.018)/3 = 0.023$

C_{crit} 0.27
 Cut off value $0.023 + 0.27 = 0.293$

Interpretation of Result

Divide the absorbance of a sample by the cut off value

Nonreactive Results

If the result is less than 1.0, the sample is reported as negative. No further testing is required

Reactive Results

If the result is 1.0 or more, the sample must be considered as containing antibodies to HCV. The samples should be retested in duplicates.

Note: If the value is less than 1.0 but more or equal 0.9, we recommend to retest the sample in duplicate, too.

Limitations

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings

Diagnostic Sensitivity

The diagnostic sensitivity was determined by testing a panel of 400 positive samples. The results obtained show a diagnostic sensitivity of 100 %.

Diagnostic Specificity

The diagnostic specificity was determined by testing a panel of 400 samples, in parallel with a test already available on the market. The results obtained show a diagnostic specificity of 99.5%.

Performance Characteristics

Measurement precision

Within run (Repeatability)

With one human serum based panel, using one batch of reagent in replicates of 100 samples data are as following:

Mean = 1.16 SD = 0.068 CV = 6.98 %

Run to run (Reproducibility)

With one human serum based panel, using one batch of reagent in replicates of 100 samples across 3 separate runs data are as following:

Mean = 1.04 SD = 0.028 CV = 8.78 %

References

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4. Houghton M, Weiner A, Han J, Kuo G, Choo QL. Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology. 1991;14(2):381-388.
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