

Anti-HCV ELISA

REF: 1308 001 96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the qualitative detection of Anti-HCV (Hepatitis C Virus Antibody) in human serum or plasma (3rd Generation)

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 provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins. Hepatitis C is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. The discovery and characterization of the hepatitis C virus (HCV) led to the understanding of its primary role in posttransfusion hepatitis and its tendency to induce persistent infection. 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. The major causes of HCV infection worldwide 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 persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use).

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. After the washing, in the second step, enzyme conjugate is added to the reaction mixture. During the incubation, the Anti-HCV captured to the solid phase in the first step reacts with mouse Anti-human IgG within enzyme conjugate. Then a complex is generated between the solid phase, the Anti-HCV within the sample and the mouse Anti-human IgG in the enzyme conjugate by immunological reactions. After a second washing, substrate A and substrate B are then added and catalyzed by this complex, 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

Coated Wells

1 plate of 96 wells pre-coated with recombinant HCV.

Negative Control

1 x 1 ml of phosphate buffered solution containing proteins of bovine origin. 0.05% preservative was added.

Positive Control

1 x 1 ml of phosphate buffered solution containing pooled heat-inactivated human serum and plasma positive for Anti-HCV and proteins of bovine origin. 0.05% preservative was added.

Sample Diluent

1 x 11.5 ml of Tris-NaCl buffer and Casein. 0.02% sodium azide preservatives was added.

Enzyme Conjugate

1 x 11.5 ml of horseradish peroxidase labeled mouse Anti-human IgG in a stabilizing buffer containing proteins of bovine origin. Contains 0.05% preservative.

Substrate A

1 x 7.5 ml of hydrogen peroxide.

Substrate B

1 x 7.5 ml of TMB (3, 3', 5, 5'-tetramethylbenzidine) in a buffer solution.

Wash Solution Concentrate










1 x 50 ml of 20 times working wash solution.

Stop Solution

1 x 7.5 ml of 0.62 mol/l sulfuric acid.

2 Pieces of plate lid and 1 Zip-lock bag.

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. Store all components at 2-8°C. Do not freeze. Avoid strong light.
2. Store unused wells in a sealed zip-lock bag (with desiccant provided) in the aluminum foiled pouch with a plate, as well as the unused reagents at 2-8°C under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.

Reagent Preparation

1. Bring all reagents to room temperature (18-25 °C) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
2. Adjust the incubator to 37 °C.
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.

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. The positive control contains human sourced components, which have been tested and found non-reactive for HIV 1 and HIV 2 antibodies, Anti-HTLV I & II and HCV antibodies, in addition, the negative has been tested and found non-reactive for HBsAg, while the positive control has been tested and found reactive for HBsAg. It is recommended that all human sourced materials 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. Determine the total number of wells needed for the assay. In addition to specimens, one substrate blank, three negative controls and two positive controls must be included on each plate or partial plate. Unused wells should be stored in the supplied Zip-lock bag with desiccant provided.
2. Prepare a record (plate map) identifying the placement of the blank, controls and specimens in the microwells.
3. To the wells of controls add 100 µl of the negative or positive controls.

4. Add 100 µl of sample diluent to each of the rest of the wells, then add 10 µl of sample to the wells containing sample diluent
5. Shake on a plate shaker for 30 seconds to completely mix the diluted samples within the wells.
6. Cover the plate with a lid and incubate at 37 °C for 30 minutes
7. Add 350 µl of wash solution, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add 100 µl of enzyme conjugate to each well except the blank well.
9. Cover the plate with a lid and incubate at 37 °C for 30 minutes.
10. Repeat step 7.
11. Add 50 µl of substrate A, then 50 µl of substrate B to each well, including the blank well.
12. Gently mix for 15 seconds and incubate at 37 °C in the dark for 10 minutes without shaking.
13. Add 50 µl of stop solution to each well, including the blank well.
14. Gently mix for 15 seconds. It is very important to make sure that the blue color changes to yellow completely.
15. Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize interference) in a microplate reader. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

Assay Scheme	
Controls	100 µl
Sample diluent	100 µl
Sample	10 µl
Incubation time	30 minutes
Washing step	6 times
Enzyme Conjugate	100 µl
Incubation time	30 minutes
Washing step	6 times
Substrate A	50 µl
Substrate B	50 µl
Incubation time in dark	10 minutes
Stop solution	50 µl
Read absorbance	450 nm

Test Validation

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 0.6

Negative Control

Mean absorbance of Negative Control is lower than 0.1

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 = 0.1 + Mean of the Negative Control replicates.

N.B. If the mean absorbance of Negative Control replicates < 0.05, use the following equation:

The cut-off = 0.1 + 0.05

Example

Mean Negative Control	= (0.030 + 0.022+ 0.018)/3 = 0.023
Cut-off Value	= 0.1 + 0.05 = 0.15

Interpretation of Result

Nonreactive Results

Samples giving an absorbance less than the cut-off value are considered nonreactive.

Reactive Results

Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay. Such samples should be retested in duplicate. Samples that are reactive in at least one of the retests are considered positive for Anti-HCV and should be confirmed by using a confirmatory kit. Samples that are nonreactive in both wells on retest should be considered nonreactive.

Limitations

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.

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 10040 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

1. Alter HJ. The dominant role of non-A, non-B in the pathogenesis of post-transfusion hepatitis: a clinical assessment. *Clin Gastroenterol.* 1980;9:155-170.
2. Esteban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. *Lancet.* 1989;2(8658)
3. Zeldis JB, Jain S, Kuramoto IK, et al. Seroepidemiology of viral infections among intravenous drug users in northern California. *West. J. Med.* 1992;156(1):30-35.
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.
5. Van der Poel CL, Cuyper HTM, Reesink HW, et al. Confirmation of hepatitis C virus infection by new four -antigen recombinant immunoblot assay. *Lancet.* 1991;337:317-319.



Egyptian Company for Biotechnology (S.A.E)

Obour city industrial area. block 20008 piece 19 A. Cairo. Egypt.

Tel: +202 4665 1848 - Fax: +202 4665 1847

www.spectrum-diagnostics.com

E-mail: info@spectrum-diagnostics.com



IFUFES03

Rev.(3), 1/4/2015