minutes .

12. Stop the reaction by adding 100µl of stop solution. The stop solution should be added in the same sequence as substrate addition.
13. Calibrate the plate reader with the Blank well and read the absorbance at 450nm with 600-700 nm as reference within 30 min of stopping the reaction.

SUMMARY OF THE ASSAY PROCEDURE:

Add sample Diluent	200 µl
Add sample	10µl
Incubate	30 minutes at room temperature.
Wash	6 times
Add HRP-Conjugate	100µl
Incubate	30 minutes at room temperature.
Wash	6 times
Substrate	100 µl
Incubate	30 minutes at room temperature.Incubate in Dark.
Stop the reaction	100µl stop solution
Read the absorbance	450nm or 450/630 nm

ASSAY CONDITIONS

1. The individual absorbance value of Positive control should be more than 1.0 OD

INTERPRETATION OF RESULTS

Cut off value is calculated by adding 0.3 to average absorbance value of negative control.
An Example Calculation:
Mean PC is calculated as follows
Abs of PC 1 : 2.246
PC 2 : 2.304
Mean PC : (2.246+2.304) / 2 = 2.275
Mean NC is calculated as follows
Abs of NC 1: 0.020
NC 2: 0.030
Mean NC : (0.020 + 0.030) / 2 = 0.025
The cut off value = Avg of NC + 0.3 = 0.025 + 0.3 = 0.325

1. Sample with absorbance value less than cut off value is considered non reactive by LIFELISA HCV ELISA kit and are considered as negative for HCV antibodies.
2. The specimen with absorbance value greater than or equal to cut off value are considered as reactive for HCV antibodies.
3. Sample with absorbance value within 10% below the cut off should be considered suspect for the presence of HCV and should be retested in duplicate. If both duplicate retest sample absorbance value is less than cut off value, the sample is considered non reactive.

ASSAY PERFORMANCE:

Sample Data	Total Number	LIFELISA HCV	Commercial ELISA
Number of samples tested	350	350	350
Number of Negative Samples	280	280	280
Number of Positive Samples	70	70	70

Two Hundred and eighty negative samples and 70 positive samples were tested with LIFELISA HCV and compared with a commercially available ELISA kit utilizing the similar principle. The results are given below:
Based on this evaluation:
Sensitivity of LIFELISA HCV : 100%
Specificity of LIFIELISA HCV : 100%

ASSAY LIMITATIONS

1. The test should be used for the detection of HCV antibodies in serum or plasma only , not in other body fluids.
2. Though LIFELISA HCV is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HCV infection. Reactive samples should be retested with confirmatory assays like Neutralization assays ,HCV RNA by PCR etc
3. Absence of antibodies does not indicate that an individual is absolutely free of HCV infection.
4. This test should only be used as screening test and its result should be confirmed by other supplemental method before taking clinical decisions.
5. Testing of pooled sample is not recommended.
6. The assay cannot distinguish between infections with HCV.
7. This is a qualitative assay and the results cannot be used to measure antibodies concentrations.

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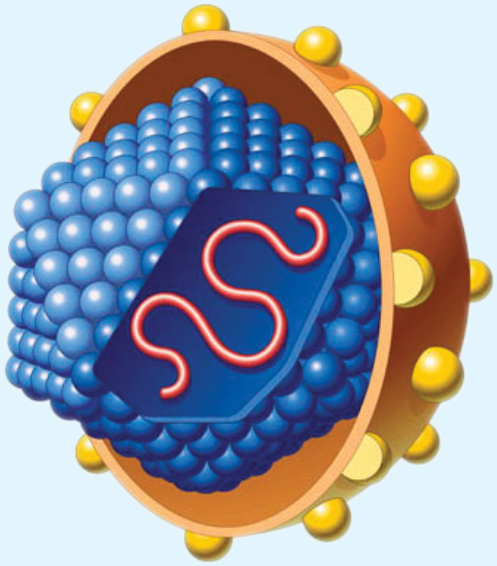

LifeLisa® HCV

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LifeLisa™ HCV

ELISA test for the detection of antibodies to
HCV in human serum or plasma
Two-Step Incubation, Indirect ELISA Principle

INTENDED USE

This LIFELISA HCV is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antibodies to Hepatitis C Virus (HCV) in human serum or plasma. It is intended for screening of blood donors and as an aid for the diagnosis of clinical conditions related to infection with HCV

SUMMARY AND EXPLANATION

Infection by Hepatitis C virus (HCV) is currently one of the most relevant public health problems, due to features such as the long period of asymptomatic infection, The Hepatitis C virus is the major cause of the disease formerly known as non-A, non-B post transfusion hepatitis. Additionally, it can become chronic in upto 85% of infected people, and it increases the risk for developing severe complications such as liver cirrhosis and liver cancer.. HCV and the appearance of detectable antibodies (window period) is generally more than 40 days (Schreiber et al, 1996; Barrera et al, 1995). HCV infection in India varies between 0.3% to 11.3%. In a large percentage of HCV cases, transmission is by transfusion and other parenteral means such as sharing of needles, occupational exposure to blood and hemodialysis. However, in case of half of HCV infections, the route of transmission is unknown.

PRINCIPLE OF THE ASSAY

HCV, a single stranded RNA virus, is a member of the family Flaviviridae. Six major genotypes (1-6) and a series of subtypes of HCV have been identified.. Genotypes 1-3 show a worldwide distribution while genotypes 4 and 5 appear predominantly in Africa and genotype 6 in Asia.

This HCV ELISA is a two step incubation "Indirect" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with highly specific synthetic peptides representing HCV antigens from the putative core and nonstructural regions NS3 , NS4 and NS5 from multiple genotypes resulted in a marked improvement in sensitivity . Patient's serum or plasma sample is added, and during the first incubation step, the specific HCV antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. Horseradish Peroxidase(HRP) conjugated Antihuman IgG is added to each well. This conjugate will bind to HCV peptide-antibody complex present. The microwells are washed to remove unbound conjugate. Substrate solution containing chromogen and hydrogen peroxidase is added to the wells and incubated. Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with Diluted Sulfuric Acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remains colorless.

KIT COMPONENTS

LIFELISA HCV has following components:

1. Coated Microwells: 96 well plate.
2. Sample Diluent: Buffer containing protein stabilizer and antimicrobial agents as preservatives.
3. Conjugate Diluent: Buffer containing protein stabilizer and antimicrobial agents as preservatives.
4. Enzyme Conjugate: Antibody tagged with HRP enzyme (50X). To be diluted 50 times in conjugate diluent.
5. Negative Control: Inactivated and stabilized human serum non reactive for HIV, HCV and HBsAg.
6. Positive Control: Inactivated and stabilized human serum reactive for HCV and non reactive for HIV and HBsAg.
7. Substrate: Ready to use TMB solution.
8. Stop solution: Ready to use diluted sulfuric acid.
9. Wash Buffer: Concentrated buffer with surfactant (20X). To be diluted 20 times in distilled water.
10. Pack Insert.
11. ELISA work sheet.
12. Plate sealer.
13. Microwell holder.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C; do not freeze. To assure maximum performance of this LIFELISA HCV kits, avoid contamination during storage. Diluted wash buffer is stable for two weeks at 2-8 °C. Diluted conjugate is stable for 4 hrs at 2-8 °C.

ADDITIONAL MATERIALS REQUIRED

1. Micro pipette and tips
2. ELISA Reader
3. ELISA Washer
4. Incubator
5. Distilled or Deionized Water
6. Disposable gloves
7. Timer
8. Clean glass ware
9. Vortex mixer
10. Adsorbent sheets
11. Disinfectants
12. Waste containers

SAMPLE COLLECTION

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matter in the sample should be removed by centrifugation. Highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. However specimen can be stored refrigerated for short duration. For long storage, freeze the sample multiple freeze-thaw cycles should be avoided. Do not heat inactivate the samples.

PRECAUTIONS AND SAFETY

This kit is intended FOR IN VITRO USE ONLY

1. Use disposable gloves and protective cloth when handling samples.
2. Do not pipette any material by mouth.
3. Do not smoke, eat and drink in the areas where sample is being handled.
4. Immediately clean up any spills with sodium hypochlorite.
5. Do not exchange reagents from different lots, or use reagents from other commercially available kits.
6. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
7. Allow the reagents and samples to stabilize at room temperature before use.
8. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
9. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
10. Never allow the microplate wells to dry after the washing step.
11. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. All specimens from human origin should be considered as potentially infectious.
14. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1 & 2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
16. The Stop solution is a strong acid. Corrosive. Use it with appropriate care.
17. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemicals, and substances like sodium hypochlorite, acids, alkalines etc. Do not perform the assay in the presence of such substances.
18. Avoid strong light exposure during the assay.
19. Thorough washing of the wells is critical to the performance of the assay. Avoid cross contamination of the wells during washing. Inadequate wash leads to false positive results by giving high background reaction. The use of an automated microwell washing system will assure consistent washing, and is recommended.
20. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals totally dissolve.

REAGENT PREPARATION

1. Sample Preparation

a) Tube Dilution: Make the tubes carefully for the proper identification of the samples. Dilute the serum sample to be tested with sample diluent (1:20 dilution) in separate tubes(200 µl diluent + 10 µl sample).Use a separate tip for each sample

b) Microwell Dilution: Pipette 200 µl of sample diluent in to the microwell, add 10 µl of serum sample to be tested. Ensure enough mixing of the sample to be tested.

2. Working Conjugate:
   Dilute concentrated conjugate 1:50 in conjugate diluent. Mix thoroughly before use. Do not store the working solution for more than 4 hrs.

No of Strips	1	2	3	4	5	6
Conjugate(50X) (µl)	20	40	60	80	100	120
Conjugate Diluent (µl)	980	1960	2940	3920	4900	5880
No of Strips	7	8	9	10	11	12
Conjugate(50X) (µl)	140	160	180	200	220	240
Conjugate Diluent (µl)	6860	7840	8820	9800	10780	11760

3. Wash Buffer:
   Dilute wash buffer 20 times with distilled water or deionized water
   (For example 5 ml of wash buffer (20X) to 95 ml of distilled water)

TEST PROCEDURE

1. Allow the reagents to reach room temperature.
2. Arrange required number of strips in microwell holder.
3. Prepare ELISA protocol sheet indicating the location of the samples and controls
4. Add 200 µl sample diluent to A-1 well as blank.Use A-2,3 wells for negative controls and A-4,5 wells for positive controls.
5. Add 200 µl sample diluent in each remaining well followed by addition of 10µl sample. (Refer Microwell Dilution). Note: Use a separate disposable pipette tip for each specimen, negative and positive control to avoid cross contamination.
6. Run duplicate for positive control and negative control.
7. Mix gently. Seal the plate with the plate sealer and incubate for 30 minutes at Room temperature.
8. At the end of the incubation, remove and discard the plate sealer. Wash each well by filling approximately 350 µl diluted wash buffer (repeat 6 times). Avoid over flow of buffer from one well to another. Each time, the microwells to be allowed to soak for 30 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it so as to remove any remaining liquids (blot dry).
9. Add 100µl of diluted conjugate to each well except in the blank and incubate for 30 minutes at room temperature.
10. Wash 6 times as in step 8. Blot dry.
11. Dispense 100µl of ready to use substrate and incubate away from light at room temperature for 30