MaxLISA HCV 3.0 (Ab) ELISA Test

(Enzyme Linked Immunosorbent Assay)

ORDERING INFORMATION

Ref. No.	Pack Size
AVELHCV3-96	96 Tests

1. INTENDED USE : MaxLISA HCV 3.0 (Ab) ELISA Test is an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies to Hepatitis C Virus in human serum or plasma. This test is not automated.

2. INTRODUCTION: 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 (HCV), which was formerly described as the parenterally transmitted non-A, non-B hepatitis. Throughout the world indicate that the virus is transmitted through sexual contact, contaminated blood and blood products, blood transfusion and other close personal contacts. The worldwide prevalence of HCV is 0.2 to 2% in blood donors and up to 80% in intravenous drug users. The nucleocapsid encoding region seems to be the most conservative among the isolates obtained all over the world. The determination of antibody to HCV has become mandatory in the screening of blood units to prevent post-transfusion hepatitis

3. TEST PRINCIPLE: MaxLISA HCV 3.0 (Ab) ELISA Test is a solid phase enzyme linked immunosorbent assay (ELISA). The combination of antigens for the structural and non-structural HCV proteins are coated onto the microwells. Samples along with positive and negative controls are added in the coated wells and incubated. The wells are washed to remove unbound components and anti-human IgG conjugated to horseradish peroxidase (HRPO) is added. After incubation the wells are washed again and bound enzyme is detected by adding substrate. An acid stopping solution is added to each well and the color is read on photometer at 450 nm and reference wavelength 620 nm is recommended.

4. KIT CONTENTS:
Store all components at 2-8°C when not in use

Material	96 Tests
Microwells (1 x 96 well microplate)	Microwells coated with HCV antigen packed in a pouch with desiccant
Sample Diluent (1 x 20 ml)	Buffer contains stabilizers and antimicrobial agents as preservative
Conjugate Diluent (1 x 20 ml)	Buffer contains stabilizers and antimicrobial agents as preservative
Enzyme Conjugate Concentrate (100X) (1 x 0.2 ml)	Anti-human IgGs conjugated with horseradish peroxidase with stabilizers
Wash Buffer (20X) (1 x 25 ml)	Phosphate buffer saline with surfactant. Dilute 1:20 with distilled water before use.
TMB Substrate A (1 x 10 ml)	To be diluted with TMB diluent before use
TMB Substrate B (1 x 10 ml)	Buffer solution containing H_2O_2
Positive Control (1 x 1 ml)	Ready to use, Inactivated and reactive for HCV and non-reactive for HIV & HBsAg
Negative Control (1 x 1 ml)	Ready to use, normal human Negative for HBsAg, HCV &HIV
Stop Solution (1 x 12 ml)	Ready to use.
Pack insert	1 No.

5. MATERIALS REQUIRED BUT NOT PROVIDED
- Distilled or Deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves

6. SAMPLE COLLECTION, STORAGE & HANDLING
- Only human serum or plasma samples should be used for the test
- While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis
- Fresh serum/plasma samples are preferred.
- Serum and plasma (EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20 to -70°C)
- Avoid repeated freezing and thawing.
- Do not use sodium azide as preservative because it inactivates horseradish peroxidase
- Microbial contaminated and hemolyzed samples may give erroneous Results.

7 . PRECAUTIONS:
- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is strongly recommended while running the test.
- Before performing the test, read all the instructions carefully and follow each and every instruction to get the intended and accurate results.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Use the required volume of specimen while testing.
- Do not let the strips dry in between the steps.
- Follow GLP and biosafety guidelines for handling and disposal of potentially infective materials/expired kit/used kits.
- Carefully read and follow the assay procedure and storage instructions. Deviation will lead to erroneous results.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact wit heyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
- In case of performance changes or product malfunction, stop using the kit immediately and contact your local distributor.
- Controls and samples to be tested should be handled as potentially hazardous as they are capable of transmitting infection.

8. PREPARATION OF REAGENTS
Note: Before use, allow reagents and samples at room temperature (20-30°C).

8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ± 2-8°C

Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.
8.1.2. Negative control
8.1.3. HCV positive control
8.1.4. Sample Diluent
8.1.5 Conjugate Diluent
8.1.6. Stop solution

8.2. Reagents to reconstitute:
8.2.1. Wash buffer (20X):

8.2.1. Wash buffer (20X):
- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals Dissolve.
- Dilute 1:20 in distilled water to obtain the ready to use washing solution. Mix 25 ml of 20X wash buffer concentrate with 475 ml of distilled or deionized water. Working wash buffer is stable for 2 months when stored at 2-8°C.

8.2.2. Wash Procedure:
- Incomplete washing will adversely affect the test outcome.
- Aspirate the well contents completely into a waste container.
- Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another well.
- Aspirate completely and repeat the wash procedure for a total 5 times of washes.
- Automated washer if used should be well adjusted to fill each well completely.
- Tap plate on absorbent papers till no droplets appear on the paper

8.2.3. Preparation of working conjugate:
Make a 1:100 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use. Determine the quantity of working conjugate solution to be prepared from table given below.
Mix solution thoroughly before use

Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Enzyme Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:
Mix TMB Substrate A and TMB Substrate B in 1:1 ratio to prepare working substrate buffer 5 to 10 minutes before use. Avoid exposure to light.

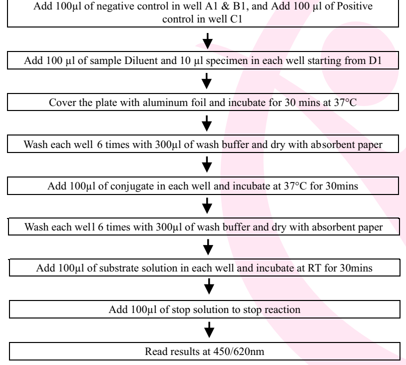
Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate A (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Substrate B (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

9. TEST PROCEDURE:

I. Bring all the reagents and specimen to room temperature before use.
II. Take the required number of strips and fix them to frame and immediately close the pouch.
III. Prepare template in data sheet indicating the location of controls and Specimens.
IV. Add 100 µl of negative control in each well no. A1& B1
V. Add 100 µl positive control in C1 well.
VI. Add 100µl of sample diluent in each well starting from D1 respectively.
VII. Add 10µl of specimen in each well starting from D1
VIII. Cover with aluminum foil and incubate for 30 minutes at 37°C.
IX. Before 5 to 10 minutes of incubation, make a 1:100 dilution of conjugate with conjugate diluent.
X. After incubation, aspirate the contents from all the wells and wash each well 6 times with by filling approximately 300µl diluted wash buffer.
XI. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100 µl of prepared diluted conjugate into each well.
XII. Incubate the plate at 37°C for 30 minutes.
XIII. Before 5 to 10 minutes of incubation, make a 1:1 dilution of substrate with substrate buffer.
XIV. Aspirate and wash as described in step no.11.
XV. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted substrate into each well and incubate at room temperature for 30 minutes.
XVI. Add 100µl of stop solution each well.
XVII. Read absorbance at 450/620nm within 10 minutes in ELISA READER.

10. Flow chart for Test Run criteria:



11.Calculation of the cut-off value:

A. Negative control means (NCx)

Absorbance of Negative control (NC)	
NC1	0.071
NC2	0.075
Mean (NCx)	**(0.070+0.075)/2 = 0.073**

B. **Cut off value:** NCx+0.220 = 0.073 + 0.220 = 0.293

C. **Quality Control:**
Results of an assay are valid if the following criteria for the controls are met:
PC must be ≥ 0.500.
NC must be ≤ 0.200.

D. **Abbreviations:**
NC - Absorbance of Negative control
NCx - Mean Negative control
PC - Absorbance of the Positive control

12. Interpretation of Results:

- Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for HCV.
- Test specimens with absorbance value greater than or equal to the cut off value are reactive for HCV by MaxLISA HCV 3.0 (Ab) ELISA Test.
- Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the criteria of MaxLisaHCV. If the samples are considered positive, the test should be conducted two or more times.
- If any one of positive duplicates retest sample absorbance value is equal to or greater than the cut off or both duplicate retest values are equal to or greater than the cut off, the specimen is considered reactive by the criteria of MaxLISA HCV 3.0 (Ab) ELISA Test. Further confirmation by other confirmation assays including western blot or PCR is recommended.

13. Limitations:

- The test should be used for detection of HCV in serum or plasma only and not in other body fluids.
- MaxLISA HCV 3.0 (Ab) ELISA Test is a screening assay, it's not be used as a sole criterion for diagnosis of HCV infection.
- Reactive sample should be retested with confirmatory assays like western blot or by PCR.
- The most common assay mistakes are: using kits beyond the expiry date, wrong washing procedures, contaminated reagents, incorrect assay procedure steps, failure to add specimens or reagents, timing errors, the use of incompletely clotted serum specimens.
- This assay cannot be utilized to test pooled (mixed) plasma. MaxLisa-HCV has been evaluated only with individual serum or plasma specimens.
- MaxLISA HCV 3.0 (Ab) ELISA Test is a qualitative assay and the results cannot be used to measure antigen concentration.

14. PERFORMANCE CHARACTERISTICS

The performance of 3rd Generation MaxLISA HCV Test is evaluated in-house with fresh as well as frozen samples from low risk to high risk groups by using a panel containing 645 nos. sample (110 Positive and 535 Negative). Compare with other commercially available ELISA kits. The sample from random blood donors, patients with diseases related to HCV and patients with diseases unrelated to HCV. The result of all the sera with a defined HCV status were fully comparable with those of 3rd generation MaxLISA HCV Test. The result of in-house study are as below table:

Type of Specimen	Observation				
	MaxLISA HCV Test		Commercial available HCV ELISA		Total Results
	Positive	Negative	Positive	Negative	
Positive	110	0	110	0	110
Negative	01	534	0	535	535

On the basis of above internal evalutions,
The Sensitivity is 100%
The Specificity is 99.8%

Precision
Intra-Assay
Within-run precision has been determined by using 10 replicates of four specimens: a negative, a low positive, medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99% of the time.
Inter-Assay
Between-run precision has been determined by 10 independent assays on the same four specimens: a negative, a low positive, medium positive and a high positive. Four different lots of the MaxLISA HCV 3.0 (Ab) ELISA Test have been tested over a 10 Days period using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified 99.8% of the time.

15. INTERFERING SUBSTANCES

The following potentially interfering substances were added to Syphilis negative and positive specimens.

Acetaminophen:	20 mg/dL	Caffeine:	20 mg/dL
Acetylsalicylic Acid:	20 mg/dL	Gentisic Acid:	20 mg/dL
Ascorbic Acid:	2g/dL	Albumin:	2 g/dL
Creatin:	200 mg/dL	Hemoglobin	1.1 mg/dL
Bilirubin:	1g/dL	Oxalic Acid:	600mg/dL

None of the substances at the concentration tested interfered in the assay.

16. References:

- World Health Organization: Advance in Viral Hepatitis, Technical report series no.602 (1977), Geneva. Voller A., Bidwell D.E. and Barriett A: The enzyme linked immunosorbent assay (ELISA), Dynatech Europe: Borough House, Ru du pre, Guernsey, G.B. (1979).
- NCCLS Document I 17-P (1991). Protection of laboratory workers for instrument biohazards. Vol.1(15).

Symbols Used on Pack

Symbol	Meaning	Symbol	Meaning
REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
Σ	Contains sufficient no. of test		

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