20. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

21. REFERENCES

1. Caypers, H.T.M. et. al. (1971) J. of Hepatology,13, 5.15.
2. Sayers, M.H. & Gretch D.R. (1993). J. Transfusion 30,809-13.
3. Halfon, P. et. al. (1997) J. Medical Virology. 52:391-395
4. Sarin, S.K. & Hess. G. (1998). Transfusion associated Hepatitis. CBS Publishers, New Delhi.
5. S. Osborne, E. Cecconato, S. Griva, F. Garetto, R. Calogero, C. Rosa and F. Bonelli : Expression in E. coli and purification of a chimeric p22-NS3 recombinant antigen of Hepatitis C Virus (HCV). Federation of European Biochemical Societies, Volume 324, number 3, 253-257.

22. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod provided with the kit.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay
	g) Improper preparation of enzyme conjugate (dilution error) improper mixing of reagents.	Check procedure & repeat assay
	h) Kit expired/ deteriorated/ reagent of different kit used.	Check storage and expiry of the kit before use and should be stored at 2-8°C.
2. High O.D. value of Negative control	a) Plate not stopped after 30 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
	b) Same microtip used for Positive and negative controls	Change micropipette tips while addition of negative/ positive control
	c) Nonspecific attachment/ binding of other reagent while addition of next step.	If plates get scratches/ aberrations during washing, non specific proteins may bind while addition of next step.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent due to blockage of probes ii) Filling volume not sufficient. iii) Insufficient no. of wash cycles. iv) Contaminated wash device	Check wash device and clean probes of manifold. fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously
	e) Use of wash solution from other manufacturer.	Use only HCV Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clean the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
	d) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. False Positive	a) Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
	b) High incubator temperature, incorrect timing or pipetting	Check incubator temperature, procedure & repeat assay.
	c) Use of turbid/ lipaemic or hemolyzed sample. with clear sample.	Centrifuge the sample at 5000 rpm for 30 minutes and re-run the test
6. False Negative/ low O.D. of Positive control & positive sample	a) Inadequate addition of substrate/conjugate solution.	Follow the procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) White particles in working substrate solution. again in fresh tube.	Discard the substrate and prepare the working substrate
	d) Uncalibrated pipettes, improper pipetting. carefully without bubbling.	Use only calibrated pipettes with well fitted tips & pipette
	e) Deterioration of Enzyme conjugate Conc.	Check storage of Enzyme conjugate Conc. It shall be stored at 2-8°C.
	f) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.
	h) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay.
	i) Kit deterioration	Check storage of kit and it should be stored at 2-8°C.
	j) Sample deterioration due to improper storage and / or microbial contamination.	Store the sample at 2-8°C / -20°C as recommended in the specimen collection & handling.

in vitro diagnostic Reagent, not for medicinal use

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R-00
VER-03
MN/ECV/016
Rev. Date: Jan-26

HCV Microlisa

Microwell ELISA Test for the Detection of Antibodies to Hepatitis C virus in Human Serum/ Plasma

1. INTENDED USE

The 3rd generation HCV Microlisa is an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies against HCV (anti-HCVs) in human serum or plasma. The kit is basically intended to screen blood donations to identify and eliminate the infected units of blood and for clinical diagnostic testing.

2. PRINCIPLE OF THE ASSAY

The 3rd generation HCV Microlisa is based on a highly sensitive technique, Enzyme Linked Immunosorbent Assay which detects antibodies against HCV in human serum and plasma. The HCV proteins are present in serum at levels well below the limits of detection. Thus, immunodiagnosis of HCV infection is based on detection of host generated antibodies (**anti-HCVs**) to viral proteins.

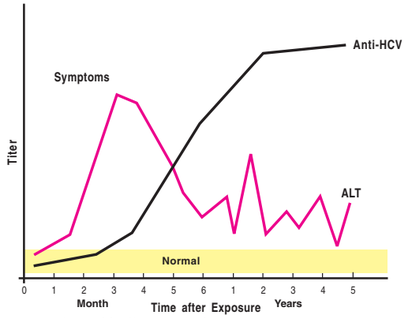


Fig. 1 Hepatitis C Virus Infection (Typical Serologic Course)

The 3rd generation HCV Microlisa utilises a combination of antigen with the sequence of both HCV structural and non-structural antigen i.e. CORE, NS3, NS4 and NS5. It has an obvious advantage over the available 2nd generation and 1st generation ELISA with improved sensitivity and specificity.

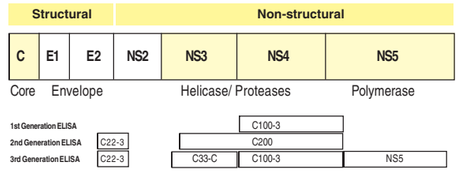
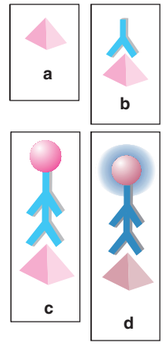


Fig. 2 Hepatitis C Virus Genome and Antigens used.

The combination of antigens for the structural and non-structural HCV proteins are coated onto the microwells (Fig.a).
Diluted sample and controls are then incubated. Antibodies to HCV, if present, bind to the immobilized HCV antigens on the microwell during this incubation period (Fig. b).



The microwells are then thoroughly washed with the diluted wash buffer to remove excess of unbound anti-HCV or other human IgGs which may interfere with the test. An enzyme conjugate, anti-human IgG conjugated with HRPO is added. The excess of enzyme conjugate is again removed with diluted wash buffer. At this stage the microwells hold only the bound antigen-anti HCV-enzyme conjugate complex (Fig. c).

In the next step, the freshly prepared substrate solution is incubated with the complex in the microwells. The enzyme substrate reaction leads to development of a blue colour which is indicative of the Ag-Ab reaction which has occurred in the microwell. In the final step the Stop Solution is added and the optical density of the developed colour is read photometrically (Fig.d.)

3. KIT PRESENTATION

- 96 Tests

4. KIT & ITS COMPONENTS

Microwells	Breakway microwells coated with HCV antigens packed in a sealed pouch with dessicant.
Sample Diluent	Buffer containing protein stabilizers and antimicrobial agents as preservative.
Enzyme Conjugate Concentrate (100x)	Anti-human IgGs conjugated with horseradish peroxidase with protein stabilizers.
Conjugate Diluent	Buffer containing protein stabilizers.
Wash Buffer Concentrate (25x)	PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	To be diluted with TMB diluent before use.
TMB Diluent	Buffer solution containing H_2O_2 with preservative
Control —	Ready to use, normal human serum negative for antibodies against HCV, HIV-1& HIV-2 and HBsAg, containing sodium azide as preservative.
Control +	Ready to use, inactivated and diluted human serum; Reactive for HCV antibodies, non-reactive for HIV-1, HIV-2 and HBsAg containing sodium azide as preservative.
Stop Solution	Ready to use, 1N sulfuric acid.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

5. STORAGE AND STABILITY

Store all components at 2-8°C when not in use. Expiry date on the kit indicates the date beyond which the kit should not be used.

6. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Disinfectant solution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves
- Glassware

7. SPECIMEN COLLECTION & PREPARATION

1. Only human serum should be used for the test. While preparing serum samples, remove the serum form the clot as soon as possible to avoid hemolysis. Fresh serum samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
3. Do not use heat inactivated samples as their use may given false results. Haemolyzed and Icteric hyperlipemic samples may give erroneous results.

8. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

HCV Microlisa test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (10,000 rpm for 15 min.)

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

9. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in European Standard EN ISO 15223-1:2021.

Manufactured By	In vitro diagnostic medical device
No. of tests	Instruction for use
Lot No. Batch Number	Temperature Limitation
Manufacturing Date	Caution, see instruction for use
Expiry Date	Catalogue Number
Keep away from sunlight	Do not use if package is damaged
Contains biological Material of Human Origin	Contains biological Material of Animal Origin
Country of Manufacture	Keep Dry

10. WARNING & PRECAUTION

CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.

2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. 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 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. 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 with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Controls and Sample diluent contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. 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.
11. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

11. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Bring all the reagents & samples to room temperature (20-30ºC) before use.
3. Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
4. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
5. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
8. Use a separate tip for each sample and then discard it as biohazardous waste.
9. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
10. Do not allow microwells to dry once the assay has started.
11. Run negative and positive controls in each assay to evaluate validity of the kit.
12. Incubation time should not vary by more than $\pm$ 2 min.
13. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
14. Distilled or deionised water must be used for wash buffer preparation.
15. Thorough washing of the wells is critical to the performance of the assay. Overflowing of reagents or washing to adjacent wells must be prevented during washing, which may lead to incorrect results due to carry over effect.
16. Take care while preparing working substrate solution as vials of TMB Substrate & Diluent are of same size.
17. Prepare working substrate solution just 10 minutes prior to adding in the wells.
18. If blue colour or white particles appear in working substrate solution then do not use it. Take fresh containers and tips and prepare it again.
19. Use separate tips for TMB Substrate and TMB diluent.
20. Avoid strong light exposure during the assay.
21. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
22. If available, a microwell reader which contains a reference filter with settings at 620 or 630 nm should be used. Use of a reference filter minimises interference due to microwells that are opaque, scratched or irregular. However, if a reference filter is unavailable, the absorbance may be read at 450 nm without a reference filter.
23. In case of any doubt the run should be repeated.

12. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30ºC) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8ºC after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator at 37ºC.

12.1. HCV Microlisa strips:
Bring foil pack to room temperature (20-30ºC) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that one negative and three positive controls should be included in the run while opening the fresh kit. However for one or two strips, one negative and two positive control and for more strips at least one negative and three positive control should be included in each subsequent runs.

b. Unused wells should be stored at 2-8ºC, with dessicant in a aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8ºC from the date of opening of sealed pouch, when stored with desicant along with clamp & rod.

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

12.2. Sample Preparation:
MICROWELL DILUTION:
a) Pipette 100μl of sample diluent in to the microwell.
b) Add 10μl of serum sample to be tested.
c) Ensure thorough mixing of the sample with the sample diluent.

Note: Cryoprecipitate may appear in sample diluent. Please ignore them as they do not interfere with the working of the kit and result interpretation

12.3. Preparation of Wash Buffer:
a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37ºC until all crystals dissolve.

b) Prepare at least 50ml. (2 ml. concentrated buffer with 48 ml. distilled or deionised water) of buffer for each HCV Microlisa strip used. Mix well before use.

Alternatively, mix 20ml. of 25X wash buffer concentrate with 480 ml. of distilled or deionized water. **Wash buffer is stable for 2 months when stored at 2-8ºC.**

12.4. Preparation of Working Conjugate:
Dilute conjugate concentrate 1:100 in conjugate diluent. **Do not store working conjugate.** Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working conjugate solution to be prepared from table below. Mix solution thoroughly before use.

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

Note: In case any precipitate is found in conjugate diluent, it should be allowed to settle and the supernatant can be used for the test. The precipitate does not interfere with the working of the kit.

12.5. Preparation of working substrate solution :
Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

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 (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

13. WASH PROCEDURE:

1. Incomplete washing will adversely affect the test outcome.
2. 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 and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 5 additional times for a total of 6 washes.
3. Automated washer if used should be well adjusted to fill each well completely without over filling.
4. Tap upside down on absorbant sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

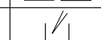
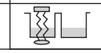
14. TEST PROCEDURE

Note: Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the stripholder with the required number of HCV Microlisa strips. **The instructions of the procedure must be strictly followed.** Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon ELISA reader software.

1. Add 100μl Negative Control in well No. A-1. Negative control is ready to use & hence no dilution is required.
2. Add 100μl Positive Control in B-1, C-1 & D-1 wells. Positive control is ready to use & hence no dilution is required.
3. Add 100μl Sample Diluent in each well, starting from E-1 well followed by addition of 10μl sample. (Refer **MICROWELL DILUTION**)
4. Apply cover seal and incubate at 37ºC $\pm$ 2ºC for 30 mins. $\pm$ 2 minutes.
5. While the samples are incubating, prepare working wash solution and working conjugate as specified in "preparation of reagents".
6. After the incubation is over, wash the wells 6 times with working wash solution according to the wash procedure given in the previous section.
7. Add 100 μl of Working Conjugate Solution in each well.
8. Apply cover seal and incubate at 37ºC $\pm$ 2ºC for 30 mins. $\pm$ 2 minutes.
9. While conjugate is incubating, prepare substrate solution in last 5 minutes of incubation as specified in "preparation of reagents". **Protect from light**.
10. Aspirate and wash the wells after incubation as described in step no. 6.
11. Add 100 μl working substrate solution in each well.
12. Incubate at room temperature (20-30ºC) in dark for 30 minutes.
13. Add 100 μl of stop solution.
14. Read absorbance at 450 nm and 630 nm in ELISA READER.

15. SUMMARY OF PROCEDURE

Dilute Serum Sample		Sample 10μl Sample Diluent 100μl
Add control (RTU)*		100μl
Cover the plate & incubate		30 mins. at 37ºC
Wash		6 Cycles
Prepare working conjugate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 Enz. conc. (μl) 10 20 30 40 50 60 70 80 90 100 110 120 Diluent (ml.) 1 2 3 4 5 6 7 8 9 10 11 12
Add Conjugate		100μl
Cover the plate & incubate		30 mins. at 37ºC
Wash		6 Cycles
Prepare Chromogenic Substrate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100μl
Incubate in dark		30 mins.
Add Stop Solution		100μl
Read Results		450 nm./630 nm.

* RTU - Ready to use

16. CALCULATION OF RESULTS

Abbreviations
NC - Absorbance of the Negative Control
PC - Absorbance of the Positive Control
PCx - Mean Positive Control

TEST VALIDITY:
Positive Control Acceptance Criteria:
PC or PC$\bar{x}$ must be > 0.5. If it is not so, the run is invalid and must be repeated.

	-	1. 897	B1 Well
PC	-	1. 855	C1 Well
	-	1. 858	D1 Well
Total		5. 610	
PC$\bar{x}$ =	5.610/3	= 1.870	

Negative Control Acceptance Criteria:
NC must be < 0.150. If it is not so, the run is invalid and must be repeated.

CUT OFF VALUE
The cut-off value is calculated as below:
Cut off Value = PC$\bar{x}$ X 0.23
e.g. PC$\bar{x}$ = 1.87 then
Cut off Value = 1.87 X 0.23 = 0.430

17. INTERPRETATION OF RESULTS

1. Test specimens with absorbance value less than the cut-off value are non-reactive for Anti-HCV.
2. Test specimens with absorbance value greater than or equal to the cut-off value are reactive for Anti-HCV.
3. Test specimens with absorbance value within 10% below the cutoff should be considered suspect for the presence of antibodies and should be retested in duplicate.
4. Specimens with absorbance value equal to or greater than the cut-off value are considered initially reactive. Original specimen should be retested in duplicate.
5. If both duplicate retest sample absorbance value is less than cutoff value, the specimen is considered nonreactive.
6. If any one of the duplicate retest sample absorbance value is equal to or greater than the cutoff or both duplicate retest value are equal to or greater than the cutoff, the specimen is considered reactive by the criteria of HCV Microlisa. Further confirmation by other EIA assays or confirmation assays (PCR) including RIBA is recommended.
7. Specimens which are not repeatedly reactive, may have shown colour due to:

a) Carry over of a highly reactive sample due to contamination of pipette tips.
b) Substrate contamination
c) Inadequate wash or aspiration during wash procedure.

18. LIMITATIONS OF THE ASSAY

1. The 3rd generation HCV Microlisa detects anti-HCV in human serum or plasma and is **only a screening test.** All reactive samples should be confirmed by supplemental assays like RIBA. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with above procedure.
2. The assay is only valid for serum and plasma from individual bleeds and not for pools of serum or plasma or other body fluids.
3 The 3rd generation HCV Microlisa detects IgG type of anti-HCV. Therefore, samples from acute HCV infection may produce a false negative result due to absence of the same as IgG type of anti-HCV are produced later during infection.
4 A non-reactive result does not exclude the possibility of exposure to or infection with HCV.
5 For the best results, the usage and storage instructions should be strictly followed. Any deviation from the procedure may lead to incorrect results.
6 A repeatable false reactive results may occur due to non-specific binding of the sample or conjugate to the well.
7 The presence of anti-HCV does not imply a Hepatitis C infection but may be indicative of recent and / or past infection by HCV.
8 Patients with auto-immune liver diseases may show false reactive results.

19. PERFORMANCE CHARACTERISTICS

(i) Performance of **3rd Generation** HCV Microlisa with reference to sensitivity and specificity has been determined by W.H.O., Geneva. The samples included in the panels for evaluation were from Latin American, Asian, European and African origin. The panels also included various sero conversion panels from Boston Biomedica Inc. (BBI), world wide performance panel and anti-HCV low titre performance panel. The evaluation indicate the following sensitivity and specificity:

Sensitivity: 100% **Specificity: 97.4%**

Ref.: WHO evaluation report dated 13th June 2001.

(ii) The performance of **3rd Generation** HCV Microlisa is also evaluated by reference Medical college/ Institute as mentioned below:

	Sensitivity	Specificity
CMC Vellore, Chennai, India:	**100%**	**98.6%**
Centre for liver disease, Hyderabad, India:	**100%**	**100%**

This information is provided for the Scientific Community Enquiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional purpose.

(iii) The performance of **3rd Generation** HCV Microlisa is also evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1148 nos. of known serum samples (including 550 tough sera). Rigorous tests were done with samples from random blood donors, patients with diseases related to HCV and patients with diseases unrelated to HCV. The results of all the sera with a defined HCV status were fully comparable with those of **3rd Generation** HCV Microlisa. The results of the in-house study done are as follows:

No. of Samples	Status	HCV Microlisa + ve	HCV Microlisa - ve
407	All RIBA +ve	407	-
741	EIA -ve	2	739

Sensitivity : 100% (407/407 RIBA Positive sera)

Specificity : 99.73% (739/741 EIA Negative sera)

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - two HCV negative and eight HCV Positive (1 strong positive and 7 weak positive). The C.V. (%) of all the ten samples were within 10%.