23. 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 deterioration	Check storage of the kit 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 Gen4 Ag & Ab 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) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
	d) Too long time for addition of controls, samples or reagents, Inconsistency in time intervals.	Develop consistent and uniform technique.
	e) 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.	Centrifuge the sample at 5000 rpm for 30 minutes and re-run the test with clear sample.
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.	Discard the substrate and prepare the working substrate again in fresh tube.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate Conc.	Check storage of Enzyme conjugate Conc. It shall be stored at 2-8°C.
	f) Deteriotion of working antigen or improper preparation of working antigen	Check reconstitution procedure and storage. It should be used within 60 days at -20°C and freeze thaw should not be more than 6 times.
	g) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes.	Follow the test procedure meticulously.
	h) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.
	i) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay.
	j) Kit deterioration	Check storage of kit and it should be stored at 2-8°C.
	k) 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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HCV Gen4 Ag & Ab Microlisa

Microwell ELISA Test for the Detection of HCV Core antigen and anti-HCV antibodies in Human Serum/ Plasma

1. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C is an infection caused by Hepalitis C virus (HCV). HCV is an enveloped, positive sense single stranded RNA virus belonging to the family flaviviridae. HCV infection can be accute and chronic that leads to liver infection and damage.

HCV antigen and anti-HCV antibodies can be screened in individuals through blood test post infection. Antibody development against viral protein may take time (upto 6 months) and infected individual may spread infection in that period. So, the combination assay for screening of both anti-HCV antibodies and HCV Core antigen can reduce the window period and improve detection of infection.

2. INTENDED USE

HCV Gen4 Ag & Ab Microlisa is an in vitro qualitative enzyme immunoassay for the detection of anti-HCV antibodies and core antigen in human serum or plasma. It is intended for screening of blood donors and for clinical diagnostic testing.

3. PRINCIPLE OF THE TEST

HCV Gen4 Ag & Ab Microlisa test is a 4th Generation qualitative enzyme immunoassay.

HCV recombinant antigen (NS3, NS4, NS5 and Core) and anti-HCV core antibodies are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. The plate is then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated to Anti-human IgG and anti-HCV antibodies is added to each well. This conjugate will bind to HCV antigen-antibody or anti-HCV antibody-antigen complex present. Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of anti-HCV antibodies and/or HCV core antigen present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450 nm. If the sample does not contain anti-HCV antibodies and/or HCV core antigen then enzyme conjugate will not bind and the solution in the wells will be either colourless or only a faint background colour develops.

4. 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	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. PACK SIZE

- 96 Tests

6. STORAGE AND STABILITY

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

7. KIT & ITS COMPONENTS

HCV Gen4 Ag & Ab Microlisa Strip Plate	Breakway microwells coated with HCV NS3, NS4, NS5 and Core antigen and anti-HCV core antibodies packed in a pouch with dessicant.
Sample Diluent	Buffer containing protein stabilizers and antimicrobial agents as preservative.
Enzyme Conjugate Concentrate (11x)	Anti-human IgG and anti-HCV antibodies labelled with horseradish peroxidase with protein stabilizers.
Conjugate Diluent	Buffer containing stabilizers and preservatives.
Wash Buffer Concentrate (25x)	PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	To be diluted in TMB Diluent before use.
TMB Diluent	Buffer solution containing H_2O_2 with preservative.
Control —	Ready to use, normal human serum negative for HCV antibodies and antigen, HIV-1& HIV-2 antibodies and HBsAg, containing sodium azide as preservative.
Control-1 +	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.
Control-2 +	HCV core antigen positive control, reconsititute with 1 ml of distilled water before use.
Stop Solution	Ready to use, 1N sulfuric acid.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

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

9. SPECIMEN COLLECTION & PREPARATION

1. Only human serum or plasma samples 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/plasma 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. Use of heat inactivated, icteric hyperlipemic and hemolyzed and Icteric hyperlipemic samples should be avoided as may give erroneous results.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

HCV Gen4 Ag & Ab 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. Centrifuge the sample at 10,000 rpm for 15 minutes to get clear sample.

(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.

11. 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 121°C 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.

12. 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 (20-30°C) 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. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
13. Distilled or deionised water must be used for wash buffer preparation.
14. 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.
15. Prepare working substrate solution just 10 minutes prior to adding in the wells.
16. 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.
17. Use separate tips for TMB substrate and TMB diluent.
18. Avoid strong light exposure during the assay.
19. 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.
20. In case of any doubt the run should be repeated.

13. 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. Prewarm the incubator to 37ºC.

1. HCV Gen4 Ag & Ab Microlisa Strip:
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 & three positive control-1 and one positive control-2 should be included in the run while opening the fresh kit. However for one or two strips, one negative, two positive control-1 and one positive control-2 and for more strips at least one negative, three positive control-1 and one positive control-2 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.

2. Preparation of working Postive Control-2:
Gently tap the vial of positive control-2 (Lyophilized) on work bench to remove any substance from rubber cap, carefully remove the cap and add 1 ml distilled water into lyophilized antigen vial. Put the cap and let it stand for 10 minutes. Mix solution thoroughly before use. **The working antigen is stable at -20ºC for 60 days (only 6 freeze thaw of liquid antigen are allowed at -20ºC).**

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 370C until all crystals dissolve.

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

c) Mix 20 ml. 25X wash buffer concentrate with 480 ml. of distilled or deionized water. **Working Wash Buffer is stable for 2 months when stored at 2-8ºC.**

4. Preparation of Working Conjugate:
Dilute conjugate concentrate 1:11 in conjugate diluent. **Do not store working conjugate.** Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from table given 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 (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	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.

14. 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 (300-350 μl) 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 4 additional times for a total of 5 washes.
3. Automated washer if used should be well adjusted to fill each well completely without over filling
4. Tap upside down on absorbent sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

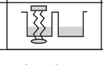
15. TEST PROCEDURE

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 Gen4 Ag & Ab Microlisa strips. The sequence of the procedure must be carefully followed. From well A-1, arrange all controls in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Add 100 μl sample diluent in each well.
2. Add 50μl Negative Control in A-1 well.
3. Add 50μl PC-1 in B-1, C-1 & D-1 wells and PC-2 in E1wells.
4. Add 50 μl sample in each well starting from F-1. **Homogenise the sample/controls and diluent with atleast one aspiration or with microplate shaker for five seconds.**
5. Apply cover seal.
6. Incubate at 37ºC $\pm$ 2ºC for 90 min. $\pm$ 5 min.
7. While the samples are incubating, prepare Working Wash Solution and Working Conjugate as specified in Preparation of Reagents.
8. Take out the plate form the incubator after the incubation time is over and, wash the wells 5 times with Working Wash solution according to the wash procedure given in the previous section (wash procedure).
9. Add 100 μl of Working Conjugate Solution in each well.
10. Apply cover seal.
11. Incubate at 37ºC $\pm$ 2ºC for 30 min. $\pm$ 2 min.
12. Aspirate and wash as described in step no. 8.
13. Add 100 μl of working substrate solution in each well.
14. Incubate at room temperature (20 - 30ºC) for 30 min. in dark.
15. Add 100 μl of stop solution and wait for 3 minutes.
16. Read absorbance at 450 and 630 nm (reference wavelength 600 - 650 nm) within 30 minutes in an ELISA READER.

16. SUMMARY OF PROCEDURE

Dilute Serum Sample		Sample Diluent 100 μl
Add control & Samples		50 μl
Cover the plate & incubate		90 mins. at 37ºC
Wash		5 Cycles
Prepare working conjugate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 Enz. conc. (ml) 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 Diluent (ml.) 1 2 3 4 5 6 7 8 9 10 11 12
Add Conjugate		100 μl
Cover the plate & incubate		30 mins. at 37ºC
Wash		5 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		100 μl
Incubate in dark		30 mins. at Room Temp.
Add Stop Solution		100 μl and wait for 3 minutes.
Read Results		450 nm./630 nm.

17. CALCULATION OF RESULTS

Abbreviations

NC	-	Absorbance (O.D.) of the Negative Control
PC-1$\bar{x}$	-	Mean Positive Control-1 absorbance
PC-1	-	Absorbance (O.D.) of the Positive Control-1
PC-2	-	Absorbance (O.D.) of the Positive Control-2

TEST VALIDITY:
Negative Control Acceptance Criteria:
NC absorbance (O.D.) must be $\leq$ 0.200. If it is not so, the run is invalid and must be repeated.

Positive Control Acceptance Criteria:
1. PC-1 absorbance (O.D.) must be $\geq$ 0.600
2. PC-2 absorbance (O.D.) must be $\geq$ 0.600

If PC-1 and PC-2 absorbance is not in defined limit, the run is invalid and must be repeated.

Result Calculation
The absorbance (O.D.) of each sample is compared with cut-off value to detect the presence or absence of antibodies to HCV or/and HCV capsid antigen.

1. Calculate the mean absorbance (O.D.) observed for HCV antibody Positive Control-1 (PC-1)
For example:
PC-1 Absorbance

-	1.012 B1 Well
-	1.023 C1 Well
-	1.015 D1 Well
Total :	3.050 3 Wells

PC-1$\bar{x}$ = 3.050/3 = 1.016

Note: If one of the HCV antibodies positive control (PC-1) individual values differs by more than 30% from the mean value, disregard that value and calculate again with the two remaining positive control values.

CUT OFF VALUE
Cut-off value is determined by using the following formula:
Cut-off Value = PC-1$\bar{x}$ X 0.27
Where PC-1$\bar{x}$ is mean absorbance (O.D) of Positive Control-1.

18. INTERPRETATION OF RESULTS

1. Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for anti-HCV antibodies and/or HCV Core antigen.
2. Specimens with absorbance value equal to or greater than the cut off value are considered initially positve by the criteria of HCV Gen4 Ag & Ab Microlisa. Original specimen should be retested in duplicate before the final interpretation.
3. Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of HCV antibodies and/or antigen and should be retested in duplicate.
4. If both duplicate retest sample absorbance value is less than cut off value, the specimen is considered negative.
5. If any one of the duplicates retest sample absorbance value is equal to or greater than the cut off or both duplicate retest value are equal to or greater than the cut off, the specimen is considered positive by the criteria of HCV Gen4 Ag & Ab Microlisa . Further confirmation by other EIA assays or confirmation assays including RIBA or PCR is recommended.
6. Specimens which are not repeatedly positive, 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.

19. LIMITATIONS OF THE ASSAY

1. HCV Gen4 Ag & Ab Microlisa assay is designed for testing antibodies against HCV and HCV Core antigen in human serum and plasma. Other body fluids and pooled samples are not recommended in this assay. Any result derived from the test of any other body fluid or from test of pooled serum/plasma may not be interpreted correctly based on the current criteria.
2. In establishing HCV infection or, in evaluating patients with Hepatitis C symptoms, HCV Gen4 Ag & Ab Microlisa testing alone cannot be used to diagnose Hepatitis C even if antibodies and/or HCV antigen are present in human serum or plasma.
3. **This is only a screening test.** A negative test result at any time does not preclude the possibility of exposure to, or infection with HCV.
4. All samples detected positive must be confirmed by using other anti-HCV ELISA/CLIA or a confirmatory test i.e. RIBA or PCR. 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 the above procedure.
5. Some samples show cross reactivity for HCV antibodies. **Following factors are found to cause false positive test results:** Naturally occurring antibodies, HIV positive, HBV positive, Dengue, syphilis, RA and anti-mouse antibodies etc.

20. PERFORMANCE CHARACTERISTICS

A) Analytical Sensitivity: The sensitivity of the kit has been determined for HCV Core Antigen using 1st WHO international standard for Hepatitis C Virus (HCV) core antigen; PEI code 129096/12 and it is equal to 1600 IU/ml.

B) In-house Evaluation:
i) Sensitivity and Specificity studies were carried out on samples fresh, as well as frozen, from low risk as well as high risk group. Performance of the test with reference to sensitivity and specificity was evaluated in-house with the panel of 91 negative samples and 28 HCV positive (6 HCV Antigen & 22 HCV Antibody) samples.

The results of all the positive and negative samples were compared with commercially available licensed test kit; Monolisa HCV Ag & Ab Ultra.

The results of the in-house study done are as follows:

No. of Samples	Status	HCV Gen4 Ag & Ab Microlisa		Monolisa HCV Ag & Ab Ultra	
		Positive	Negative	Positive	Negative
28	HCV Positive	28	-	28	-
91	HCV Negative	1	90	-	91

Sensitivity: 100% **Specificity:** 98.9%

ii) Specificity Testing:
The HCV Gen4 Ag & Ab Microlisa kit specificity is checked by using 553 clinical patient samples and 12 potentially cross-reacting specimen; HIV, HBsAg, Syphilis, RA, Dengue and ante-natal samples. The specificity on all above samples tested is 100%

iii) Performance with sero-conversion panel:
The HCV Gen4 Ag & Ab Microlisa kit performance is checked using 2 sero-conversion panels. The kit detects HCV infection earlier as compared to an anti-HCV antibody detection kit (anti-HCV kit) and results are as follows:

2 seroconversion panels (32 samples)	**anti-HCV kit**	HCV Gen4 Ag & Ab Microlisa
Number of positive samples	7	21

The sensitivity for HCV Antigen Positive samples is 100% and Specificity is also 100% on 32 samples tested.

C) The performance of HCV Gen4 Ag & Ab Microlisa has been evaluated by National Institute of Biologicals, India. The result obtained of 3 different lots are as follows:

Lot Details	Sensitivity	Specificity
LOT 1	100%	100%
LOT 2	100%	99.8%
LOT 3	100 %	100%

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of four samples: one HCV core antigen and two HCV antibody positive(one weak and one strong) and one negative samples. The C.V.(%) of all the samples were within 10%.

21. 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.

22. REFERENCES

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