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.

The combination of antigens for the structural and non-structural HCV proteins are coated onto the microwells (Fig.a). Deluted 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 Test Pack

4. KIT & ITS COMPONENTS

| Microwells   | 12 Strips (12 X 8 wells) Breakaway microwells coated with HCV antigens packed in a sealed pouch with dessicant. |
| Sample Diluent | 1 Bottle (20 ml.) 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 1 Bottle (15 ml.) Buffer containing protein stabilizers.

Wash Buffer Concentrate (25x) PBS with surfactant. Dilute 1:25 with distilled water before use.

TMB Substrate 1 Bottle (10 ml.) To be diluted with TMB diluent before use.

TMB Diluent 1 Bottle (10 ml.) Buffer solution containing H₂O₂ with preservative

Control — 1 Vial (2.0 ml.) Ready to use, normal human serum negative for antibodies against HCV, HIV-1& HIV-2 and HBsAg, containing sodium azide as preservative.

Control + 1 Vial (2.0 ml.) 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 1 Vial (15 ml.) Ready to use, 1N sulfuric acid.

Plate Sealers Adhesive backed sheets for sealing microwell plate/strip.

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.
- Vortex Mixer.
- Disposable gloves.
- Glassware.

7. 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. Do not use heat inactivated samples as their use may give false results. Hemolyzed 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 aetiology agents.

9. DESCRIPTION OF SYMBOLS USED
The following are graphical symbols used in or found on J. Mira 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 the European Standard EN ISO 15223-1:2016.
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 suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psig 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”, “Disinfection of Laboratory Sink Drains to remove Azide salts” (Center 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 (20-30°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. Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
7. Use a separate tip for each sample and then discard it as biohazardous waste.
8. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
9. Do not allow microwells to dry once the assay has started.
10. Run negative and positive controls in each assay to evaluate validity of the kit.
11. Incubation time should not vary by more than ± 2 min.
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. Take care while preparing working substrate solution as vials of TMB Substrate & Diluent are of same size.
16. Prepare working substrate solution just 10 minutes prior to adding in the wells.
17. 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.
18. Use separate tips for TMB Substrate and TMB diluent.
19. Avoid strong light exposure during the assay.
20. 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.
21. 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.
22. In case of any doubt the run should be repeated.

12. PREPARATION OF REAGENTS

Prepare the following reagents just before or during assay procedure. Reagents and samples should be at room temperature (20-30°C) before beginning the assay. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator to +37°C.

12.1 HCV Microlisa strips:

- Bring foil pack to room temperature (20-30°C) 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 2-8°C, with dessicant in a aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with dessicant along with clamp & rod.

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

12.2 Sample Preparation:

1. 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 37°C until all crystals dissolve.

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

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.

<table>
<thead>
<tr>
<th>No. of Strips</th>
<th>No. of Wells</th>
<th>Enzyme Conjugate Concentrate (µl)</th>
<th>Conjugate Diluent in (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>40</td>
<td>48</td>
<td>56</td>
<td>64</td>
</tr>
<tr>
<td>72</td>
<td>80</td>
<td>90</td>
<td>110</td>
</tr>
<tr>
<td>100</td>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>No. of Strips</th>
<th>No. of Wells</th>
<th>TMB Substrate (ml)</th>
<th>TMB Diluent (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>40</td>
<td>48</td>
<td>56</td>
<td>64</td>
</tr>
<tr>
<td>72</td>
<td>80</td>
<td>90</td>
<td>110</td>
</tr>
</tbody>
</table>

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 stringly 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 37°C ± 2°C for 30 mins. ± 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 & incubate at 37°C ± 2°C for 30 mins. ± 2 mins.
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 (20-30°C) in dark for 30 mins.
13. Add 100 µl of stop solution.
14. Read absorbance at 450 nm and 630 nm in ELISA READER.

15. SUMMARY OF PROCEDURE

<table>
<thead>
<tr>
<th>Dilute Serum</th>
<th>Sample</th>
<th>Sample Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add control (RTU)*</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Cover the plate &amp; incubate</td>
<td>30 mins. at 37°C</td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>6 Cycles</td>
<td></td>
</tr>
<tr>
<td>Prepare working conjugate</td>
<td>No of Strips</td>
<td>Enz. conc.</td>
</tr>
<tr>
<td>Add Conjugate</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>Cover the plate &amp; incubate</td>
<td>30 mins. at 37°C</td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>6 Cycles</td>
<td></td>
</tr>
<tr>
<td>Prepare Chromogenic Substrate</td>
<td>No of Strips</td>
<td>TMB (Substrate (ml))</td>
</tr>
<tr>
<td>Add Substrate</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>Incubate in dark</td>
<td>30 mins. at Room Temp.</td>
<td></td>
</tr>
<tr>
<td>Add Stop Solution</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>Read Results</td>
<td>450 nm, 630 nm.</td>
<td></td>
</tr>
</tbody>
</table>

* RTU - Ready to use

16. CALCULATION OF RESULTS

Abbreviations
NC - Absorbance of the negative control
PC - Absorbance of the positive control
PCx - Mean absorbance of Positive Control

TEST VALIDITY:
Positive Control Acceptance Criteria:
PC or PCx must be > 0.5. If it is not so, the run is invalid and must be repeated.

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 = PCx X 0.23
e.g. PCx = 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 cut-off value 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 cut-off 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 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 sera 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%
(ii) The performance of 3rd Generation HCV Microlisa is also evaluated by reference Medical college/ Institute as mentioned below:
   Centre for liver disease, Hyderabad, India: 100% 98.6%
   CMC Vellore, Chennai, 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:

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Status</th>
<th>HCV Microlisa</th>
<th>HCV Microlisa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>407</td>
<td>All RIBA +ve</td>
<td>407</td>
<td>0</td>
</tr>
<tr>
<td>739</td>
<td>EIA -ve</td>
<td>0</td>
<td>739</td>
</tr>
</tbody>
</table>

Sensitivity: 100% Specificity: 99.73%
(407/407 RIBA Positive sera) (739/741 EIA Negative sera)
## 22. TROUBLE SHOOTING CHART

### PROBLEM POSSIBLE CAUSE SOLUTION

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE CAUSE</th>
<th>SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No colour developed at the end of assay</td>
<td>a) Any one reagent has been added in wrong sequence.</td>
<td>Follow the procedure meticulously &amp; repeat assay.</td>
</tr>
<tr>
<td></td>
<td>b) Inactivated conjugate, improper storage of any contamination.</td>
<td>Check storage of enzyme conjugate and it should be free.</td>
</tr>
<tr>
<td></td>
<td>c) Microplate inactivated, due to improper storage</td>
<td>Keep unused strips in aluminium poly pouch with the dessicant pouch inside and properly closed with clamp &amp; rod.</td>
</tr>
<tr>
<td></td>
<td>d) Inactivated substrate, improper storage or preparation</td>
<td>Use freshly prepared substrate solution. Recheck procedure, repeat assay.</td>
</tr>
<tr>
<td></td>
<td>e) Omission of any step in test procedure</td>
<td>Follow the procedure meticulously &amp; repeat assay.</td>
</tr>
<tr>
<td></td>
<td>f) Incorrect (low) incubator temperature, timing or pipetting</td>
<td>Check incubator temperature, procedure &amp; repeat assay.</td>
</tr>
<tr>
<td></td>
<td>g) Improper preparation of enzyme conjugate (dilution error) improper mixing of reagents.</td>
<td>Check procedure &amp; repeat assay.</td>
</tr>
<tr>
<td>2. High O.D. value of Negative control</td>
<td>a) Plate not stopped after 30 minutes of adding stop solution</td>
<td>Follow the procedure meticulously &amp; repeat assay.</td>
</tr>
<tr>
<td></td>
<td>b) Same micropipot used for Positive and negative controls</td>
<td>Change micropipette tips while addition of negative/ positive control.</td>
</tr>
<tr>
<td></td>
<td>c) Nonspecific attachment/ binding of other reagent while addition of next step.</td>
<td>If plates get scratches/ aberrations during washing, non specific proteins may bind due to improper storage conjugate and it should be free from any contamination.</td>
</tr>
<tr>
<td>3. Too much colour in all wells of the plate (high background)</td>
<td>a) Contaminated substrate</td>
<td>Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.</td>
</tr>
<tr>
<td></td>
<td>b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer cont.</td>
<td>Check the container and quality of water used for dilution. Use of distilled water is preferred.</td>
</tr>
<tr>
<td></td>
<td>c) Over incubation of substrate and delay in addition of stop solution.</td>
<td>Follow the procedure meticulously.</td>
</tr>
<tr>
<td></td>
<td>d) Insufficient washing</td>
<td>Check wash device, fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously.</td>
</tr>
<tr>
<td></td>
<td>i) Washing not consistent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ii) Filling volume not sufficient.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iii) Insufficient no. of wash cycles.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iv) Contaminated wash device</td>
<td></td>
</tr>
</tbody>
</table>

### 21. REFERENCES


### 20. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as so that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, 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 manufacturer’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.

### 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%.