1. INTENDED USE
The 4th Generation HCV TRI-DOT is a rapid, visual, sensitive and qualitative in vitro diagnostic test for the detection of antibodies to Hepatitis C Virus in human serum or plasma.

The 4th Generation HCV TRI-DOT has been developed and designed with increased sensitivity for core and NS3 antibodies using a unique combination of modified HCV antigens. They are for the putative core (structural), protease/helicase NS3 (non-structural), NS4 (non-structural) and replicase NS5 (non-structural) regions of the virus in the form of two test dots “T₁” & “T₂” to provide a highly sensitive and specific diagnostic test.

2. INTRODUCTION
Hepatitis C Virus was identified in 1989 as the main aetiological agent of non-A, non-B hepatitis (NANBH) accounting for greater than 90% of post-transfusion hepatitis cases. HCV is a spherical virus of about 30-60 nm in diameter with single positive stranded RNA and is related to the family flaviviridae. It is considered to be the major cause of acute chronic hepatitis, liver cirrhosis and hepatocellular carcinoma throughout the world. It is therefore necessary to correctly diagnose Hepatitis C infection.

The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding elevated serum ALT levels and presence of anti-HCV in serum/plasma (Fig. 1).

3. PRINCIPLE OF THE ASSAY
1. HCV antigens are immobilized on a porous immunofiltration membrane. Sample and the reagents pass through the membrane and are absorbed into the underlying absorbent pad (Fig. 4).

2. As the patient’s sample passes through the membrane, HCV antibodies if present in serum/plasma bind to the immobilized antigens. In the subsequent washing step, unbound serum/plasma proteins are removed (Fig. 4).

3. In the next step, the protein-A conjugate is added which binds to the Fc portion of the HCV antibodies to give distinct pinkish purple dot against a white background at the test region (“T₁”& “T₂”). At the control region (“C”) a “Built-in Quality Control Dot” has been devised to confirm the proper functioning of the device, reagent and correct procedural application.

4. KIT PRESENTATION
10 Test Pack
50 Test Pack
100 Test Pack

5. 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 the British and European Standard EN ISO 15223-1:2016.

6. All materials used in the assay and samples should be
5. Do not pipette by mouth.
4. Tests are for
3. Do not smoke, drink or eat in areas where specimens or kit
2. Due to interchange of caps of the vials, the reagents may get
1. The use of disposable gloves and proper biohazardous clothing

8. WARNING FOR USERS

Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
Due to interchange of caps of the vials, the reagents may get contaminated. Care should be taken while handling the reagent caps to avoid cross contamination of the reagents.
Use a separate sample dropper for each sample and then discard it as biohazardous waste.
Avoid several times freezing and thawing of the sample to be tested.
Always allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface; this may contaminate the reagent.
Avoid microbial and cross contamination of reagents.
Return entire kit at 2-8°C, when not in use.

10. SAMPLE / SPECIMEN COLLECTION & STORAGE
Collect blood in a clean dry sterilized vial and allow it to clot. Separate the serum by centrifugation at room temperature.
It is recommended that FRESH samples should be used. If serum is not to be assayed immediately it should be stored at 2-8°C or frozen at -20°C. Serum may be stored at 2-8°C for upto 3 days and stored frozen at -20°C for 3 months. Only serum or plasma should be used for the test.

Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

11. SAMPLE / SPECIMEN PROCESSING
Though HCV TRI-DOT works best when used with fresh samples, however the frozen or viscous samples can also perform well if the following instructions are strictly adhered to:

A. Frozen samples
(i) Allow the sample to thaw in a vertical position in the rack. Mix the sample thoroughly. If particles are seen, allow them to settle at the bottom or if a centrifuge is available, the sample can be centrifuged at 10,000 r.p.m. for 15 minutes.
(ii) Insert the dropper just below the top surface of the sample and withdraw one drop of the sample.

B. Thick or viscous samples
Whenever possible, clear specimen should be used. However, viscous, thick or turbid samples which may sometimes take more than 40-60 seconds to flow through the membrane should be centrifuged at 10,000 r.p.m. for 15 minutes and retested on a fresh device to avoid inconsistent results.

6. KIT COMPONENTS
The kit contains sufficient reagent and devices for the number of tests as mentioned on the pack. All kit components should be stored at 2-8°C. DO NOT FREEZE KIT COMPONENTS.

HCV TRI-DOT Device
Individually quality checked, packed & sealed device. It is marked with "C" for Control Dot and "T1" & "T2" for Test Dots.

Buffer Solution
Buffer containing BSA and Sodium Azide. Ready to use.

Protein- A Conjugate
Protein- A Conjugate in liquid form containing Sodium Azide. Ready to use.

Sample Dropper
Long disposable plastic dropper provided for adding the sample.

7. STORAGE OF THE KIT
Store the entire kit at 2-8°C in the coolest and driest area available. The shelf life of the kit is 15 months from the date of manufacturing, when stored at 2-8°C. Do not use the kit beyond the expiry date. DO NOT FREEZE THE KIT COMPONENTS.

8. WARNING FOR USERS
CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT 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 of in accordance with established safety procedures and guidelines.
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. Protein-A Conjugate and Buffer Solution 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.)
C. Transportation
(i) The WHO guidelines for the safe transport of specimen (WHO/EMC/97.3) should be read carefully by the laboratory staff as these guidelines hold equally good for Hepatitis samples.
(ii) If the specimen is to be transported, it should be packed in compliance with the current Government regulations on transport of aetiologic agents.

12. BEFORE YOU START
The Buffer Solution & Protein A Conjugate provided in the kit has closed nozzle and screw cap with pin (outside). Before using Buffer Solution & Protein A Conjugate, keep the vial vertically straight and tap down gently on the working platform, so that the reagents comes down at the bottom of the vial. To orifice/puncture the closed nozzle, follow the instruction as illustrated below:

13. RECOMMENDATIONS FOR THE USER
(i) The procedural sequence of additions should be strictly adhered to avoid any discrepant results.
(ii) Bring all the reagents and specimens to room temperature (20-30°C) before beginning the test, as the immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature.
(iii) Mix each specimen thoroughly prior to use. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.
(iv) Place the required number of HCV TRI-DOT test devices at the working area.
(v) Cut open the pouch and take out the device for performing the test. Write the sample identification number to be tested on the device for correct correlation with results.
(vi) While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION. However, drops of each solution should be added in continuous stream to wet the entire area of membrane. If the sample does not soak-in within 40-60 seconds, observe the sample for any suspended particulate matter.
   If present, centrifuge the sample at 10,000 r.p.m. for 15 mins. and use a fresh device to re-run the test. Refer to “SAMPLE / SPECIMEN PROCESSING”.
(vii) All solutions and sample should be added to the CENTRE OF MEMBRANE.
(viii) For consistent results ensure FREE FALLING OF DROPS on the membrane holding the vial / dropper vertically for proper volume.
(ix) The protein A Conjugate should not be subjected to frequent temperature fluctuations.

14. TEST PROCEDURE
Step No. 1
Add 3 drops of Buffer Solution to the centre of the device.

Step No. 2
Hold the dropper vertically downwards and add 1 drop of patient's sample (50 µl serum or plasma) using the sample dropper provided. (use a separate sample dropper for each specimen to be tested).

Step No. 3
Add 5 drops of Buffer Solution.

Step No. 4
Add 2 drops of Protein- A Conjugate.

Step No. 5
Add 5 drops of Buffer Solution.

Step No. 6
Read result immediately and discard the device considering it to be potentially infectious.

IMPORTANT: It is important to allow each solution to soak in the test device before adding the next solution.

15. INTERPRETATION OF RESULTS
NON REACTIVE RESULT
1. Appearance of only one dot at the control region “C” indicates that the sample is NON-REACTIVE for antibodies to HCV. (Fig:a)

REACTIVE RESULT
1. Appearance of two dots, one at the control region “C” & other at the test region “T,” indicates that the sample is REACTIVE for antibodies to HCV. (Fig:b)
2. Appearance of two dots, one at the control region “C” & other at the test region “T,” indicates that the sample is REACTIVE for antibodies to HCV. (Fig:c)
3. Appearance of all the three dots, one each at “C” “T,” & “T,” region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)

INVALID RESULT
If no dot appears after the completion of test, either with clear background or with complete pinkish/purplish background the test indicates ERROR (Fig:e&f).

This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. The specimen should be retested on a fresh device (Refer sample / specimen processing).

IMPORTANT :
(i) Test dots “T,” & “T,” either dark or light in colour (pink) should be considered reactive for antibodies to HCV.
(ii) Sometimes, if the sample solution does not soak-in within 40-60 seconds, the sample should be observed for any suspended particulate matter; if it is present, centrifuge the sample at 10,000 r.p.m. for 15 minutes. Use a fresh device to re-run the test.
(iii) Sample found to be initially reactive by the above screening test must be repeated, if the sample is repeatedly reactive it must be confirmed by standard supplemental assay test RIBA.
16. LIMITATIONS OF THE TEST

(i) The 4th Generation HCV TRI-DOT 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.

(ii) The test is only validated for serum and plasma from individual bleeds and not for pools of serum or plasma or other body fluids.

(iii) A non-reactive result does not exclude the possibility of exposure to or infection with HCV.

(iv) It should be noted that repeated false reactive results may occur due to non-specific binding of the sample to the membrane.

(v) The presence of anti-HCV does not imply a Hepatitis C infection but may be indicative of recent and/or past infection by HCV.

(vi) Patients with auto-immune liver diseases, Renal disorders and Antenatal samples may show false reactive results.

(vii) The kit works best when used with fresh samples and when all the kit components are at room temperature (20-25°C). Samples which have been frozen and thawed several times contain particulates which can block the membrane, hence resulting in improper flow of reagents and high background colour which may make the interpretation of results difficult.

(viii) Optimum test performance depends on strict adherence to the test procedure as described in this manual. Any deviation from test procedure may lead to erratic result.

17. PERFORMANCE CHARACTERISTICS

(i) Performance of 4th Generation HCV TRI-DOT 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 panel 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.

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Status</th>
<th>HCV TRI-DOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>358</td>
<td>All RIBA +ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>1150</td>
<td>EIA -ve</td>
<td>358</td>
</tr>
</tbody>
</table>

Sensitivity : 100% (358/358 RIBA Positive sera)
Specificity : 99.8% (1148/1150 EIA Negative sera)

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - five HCV negative and five HCV Positive (1 strong positive, 2 medium and 2 weak positive). The C.V. (%) of all the ten samples were within 10%.

18. 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-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.

19. REFERENCES


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.

For in-vitro diagnostic use only, not for medicinal use