(vii) The kit works best when used with fresh samples and when all the kit components are at room temperature (20-25ºC). 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.

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

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

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.

(ii) The performance of **4th Generation** HCV TRI-DOT 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 1508 nos. of known serum samples (including 520 tough sera). The results of all the sera with a defined HCV status were fully comparable with those of **4th Generation** HCV TRI -DOT. The results of the in-house study done are as follows:

No. of Samples	Status	HCV TRI-DOT	HCV TRI-DOT
		+ ve	- ve
358	All RIBA + ve	358	-
1150	EIA -ve	2	1148

Sensitivity : 100% (358/358RIBA 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 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.

19. REFERENCES

1. Caypers, H.T.M. Wiakel, I.N. Vander Poel, C.L. etal (1971) J. of Hepatology, 13, 5.15.
2. Halfon, P. etal (1997) J. Medical Virology. 52:391-395.
3. Sarin, S.K. & Hess. G. (1998). Transfusion associated Hepatitis.
4. Sayers, M.H. & Gretch D.R. (1993). J. Transfusion 30,809-13..

in vitro diagnostic Reagent, not for medicinal use

R-05
VER-02
MN/HCD/019
Rev. Date: Sep.-25

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HCV TRI-DOT

Rapid Visual Test for the Qualitative Detection of Antibodies to HEPATITIS C Virus in Human Serum/Plasma

HCV Antigens for CORE, NS3, NS4 & NS5

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 "T_1" & "T_2" 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).

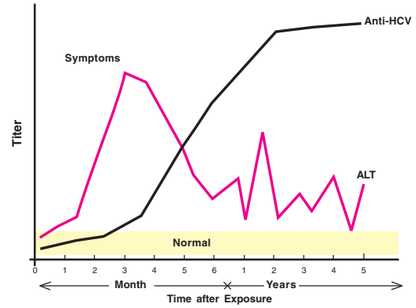

Fig.1 Hepatitis C Virus Infection
Typical Serologic Course

Recently recombinant DNA techniques have been used to encode the genome of HCV. The genome encodes for three structural proteins (capsid protein, envelope glycoproteins E1 & E2) and several non-structural proteins (NS2, NS3, NS4 & NS5) (Fig.2).

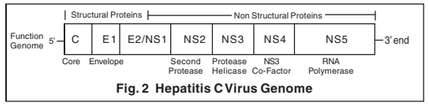

Fig. 2 Hepatitis C Virus Genome

The first generation anti HCV assay used C100-3 peptide where as the second generation assay used several recombinant viral proteins and peptides typically C-22 from the core region, C33-C from the second non-structural (NS3) region and 5-1-1 & C100-3 from the NS4 region. They were associated with a high rate of both false positive and false negative results.

This led to the development of third generation anti-HCV assay which uses a greater range of antigens from core, NS3, NS4 & NS5 regions of the HCV genome, thus providing greater sensitivity and better specificity.

Recently the **4th generation** assay for testing of anti-HCV has been established. The 4th Generation HCV TRI-DOT utilizes a unique combination of modified HCV antigens from the putative core, NS3, NS4 & NS5 regions of the virus to selectively identify all subtypes of Hepatitis C Virus in human serum/plasma with a high degree of sensitivity and specificity.

The antigens used are chemically treated and unfolded in a special way to make them more reactive & specific to different epitopes of core & NS3 region thereby minimizing the chances of crossreactivity & enhancing the specificity.

Also,the superior sensitivity of the test allows for the significantly earlier detection of antibodies during sero-conversion following HCV infection, thereby reducing the incidence of post transfusion hepatitis and providing a safer blood supply.

4th generation HCV TRI-DOT has been developed and designed using modified HCV antigens representing the immunodominant regions of HCV antigen. The device (an immuno-filtration membrane) includes two test dots "T_1" & "T_2" and a Built in Quality Control Dot "C" (Fig.3). The control dot will always develop colour during the test, thereby confirming proper functioning of the device, reagent and correct procedural application. This control dot is the "Built in Quality Control."

3. PRINCIPLE OF THE ASSAY

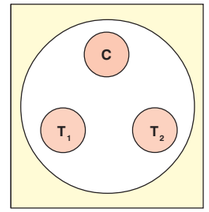

Fig. 3 Test Device

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 ("T_1"&/or "T_2"). 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.

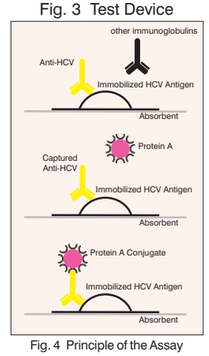

Fig. 4 Principle of the Assay

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 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

Contains biological Material of Human Origin

Contains biological Material of Animal Origin

Country of Manufacture

Keep Dry

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 2-8ºC. DO NOT FREEZE KIT COMPONENTS.

HCV TRI-DOT Device	Individually quality checked, packed & sealed device. It is marked with "C" for Control Dot and "T_1" & "T_2" 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 2-8ºC in the coolest and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. 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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
5. Tests are for in vitro diagnostic use only and should be run by competent person only.
6. Do not pipette by mouth.
7. 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 of in accordance with established safety procedures and guidelines.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. 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.)

9. PRECAUTIONS

1. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
2. 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.
3. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
4. Avoid several times freezing and thawing of the sample to be tested.
5. 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.
6. Avoid microbial and cross contamination of reagents.
7. Return entire kit at 2-8ºC, 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 2-8ºC or frozen at -20ºC. Serum may be stored at 2-8ºC for upto 3 days and stored frozen at -20ºC 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 SAMPLE

(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.

(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:

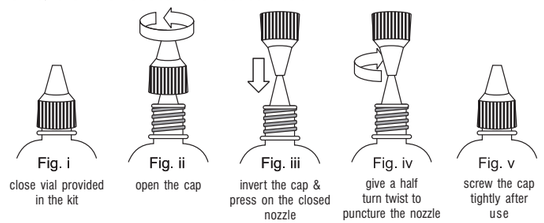

Fig. i — close vial provided in the kit

Fig. ii — open the cap

Fig. iii — invert the cap & press on the closed nozzle

Fig. iv — give a half turn twist to puncture the nozzle

Fig. v — screw the cap tightly after use

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 (20-30ºC) 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) Do not run more than 5 devices at a time.

(vii) 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".

(viii) All solutions and sample should be added to the CENTRE OF MEMBRANE.

(ix) For consistent results ensure FREE FALLING OF DROPS on the membrane holding the vial / dropper vertically for proper volume.

(x) The protein A Conjugate should not be subjected to frequent temperature fluctuations.

14. TEST PROCEDURE

Step No. 1

1. Add **3** drops of Buffer Solution to the centre of the device.

1

Step No. 2

2. Hold the dropper vertically and add **1** drop of patient's sample 50μl (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).

2

Step No. 3

3. Add **5** drops of Buffer Solution.

3

Step No. 4

4. Add **2** drops of Protein-A Conjugate directly from the conjugate vial.

4

Step No. 5

5. Add **5** drops of Buffer Solution and read results.

5

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)

a

REACTIVE RESULT

1. Appearance of two dots, one at the control region "**C**" & other at the test region "$\mathbf{T_1}$" indicates that the sample is REACTIVE for antibodies to HCV. (Fig:b)

b

2. Appearance of two dots, one at the control region "**C**" & other at the test region "$\mathbf{T_2}$" indicates that the sample is REACTIVE for antibodies to HCV. (Fig:c)

c

3. Appearance of all the three dots, one each at "**C**" "$\mathbf{T_1}$"& "$\mathbf{T_2}$" region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)

d

INVALID TEST 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).

e

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

f

IMPORTANT

(i) All initially reactive samples should be subjected to centrifugation at 10,000 r.p.m. for 15 min. It is recommended that this centrifugation step should be carried out prior to sending the sample for the confirmation on CLIA/ELISA. The test should be repeated with supernatant collected after centrifugation. If no dot appears on repetition, it indicates a falsely reactive sample. A truly reactive dot will not show much change in its colour intensity after centrifugation. The false reactivity of the sample is generally due to the presence of suspended particulate matter in the serum which may or may not be visible to the naked eye.

This critical step of centrifuging a reactive sample should be faithfully followed. Its correct application makes the test EXTREMELY SENSITIVE and completely eliminates the possibility of false reactivity.

(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) Test dots "$\mathbf{T_1}$" & "$\mathbf{T_2}$" either dark or light in colour (pink) should be considered reactive for antibodies to HCV.

(iv) In case you have any problems in our HCV TRI-DOT, please call our Technical Customer Service Cell at New Delhi Phone: +91-11-47130300, 47130500.

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.