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).

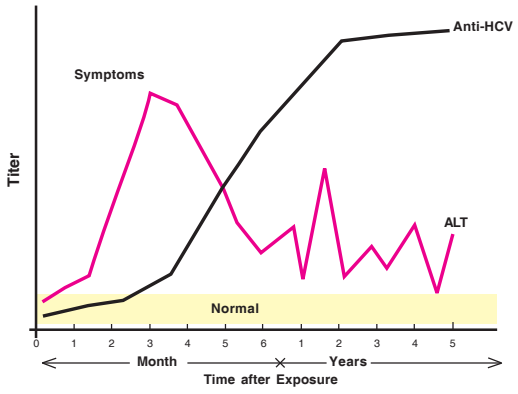


Fig.1 Hepatitis C Virus Infection
Typical Serologic Course

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

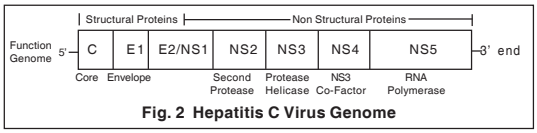


Fig. 2 Hepatitis C Virus Genome

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 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."

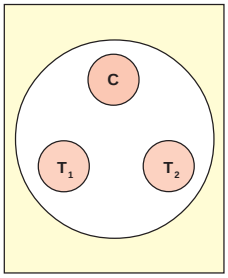


Fig. 3 Test Device

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).

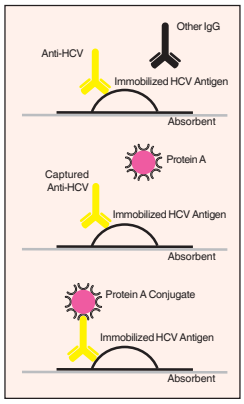


Fig. 4 Principle of the Assay

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.

4. KIT COMPONENTS

COMPONENTS	CONTENTS	PREPARATION
1. HCV TRI-DOT Test Device	Packed individually. It is marked with "**C**" for Control Dot and "T_1" & "T_2" for Test Dots.	Cut open the pouch before use.
2. Buffer Solution	Buffer containing BSA and sodium azide.	Ready to use.
3. Protein-A Conjugate	Protein-A Conjugate in liquid form containing sodium azide.	Ready to use.
4. Sample Dropper	Long Plastic dropper provided for adding the sample.	

5. STORAGE OF THE KIT

Store the kit at 2-8°C in the driest area available. **The shelf life of the kit is 15 months from the date of manufacturing.**

Do not use the kit beyond the expiry date mentioned on it. Before running the test bring all the kit components to room temperature (20-30°C) for best results. Return the entire kit to 2-8°C when not in use. DO NOT FREEZE KIT COMPONENTS.

6. KIT PRESENTATION

10 Test Pack 50 Test Pack

100 Test Pack

7. 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 is STRONGLY RECOMMENDED during the test.
2. In case there is a wound or cut in the hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. This Kit is for *in vitro* diagnostic use only.
5. All the samples to be tested should be handled as though capable of transmitting infection.
6. Spills should be decontaminated promptly with disinfectant.
7. Dispose of all specimens and materials used to perform the test appropriately using disinfectant.
8. The Protein-A Conjugate and Buffer Solution contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount 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. Thoroughly wash hands with soap after the use of this kit. In case of a needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.

8. PRECAUTIONS

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
3. 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. Place white nozzle cap on Buffer Solution vial and red cap on Protein-A Conjugate Vial.
4. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
5. Avoid several times freezing and thawing of the sample to be tested.
6. 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.
7. Avoid microbial and cross contamination of reagents.

9. 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 human 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.

10. 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.

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.

11. BEFORE YOU START

The Buffer Solution and Protein-A Conjugate vials are provided with closed nozzle and screw cap with pin(outside), then punture the nozzle before use as given below:

1. Before using reagents, keep the vial vertically straight and tap down gently on the working platform, so that reagents come down at the bottom of the vial.
2. To orifice the closed nozzle, press the inverted cap on the respective closed nozzle and give a half turn twist to ensure nozzle is properly orificed/ punctured as illustrated below in Fig. iii & iv:

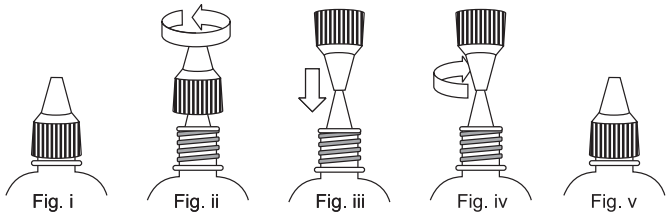


Fig. i close vial provided in the kit

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

12. ASSAY PROCEDURE

Take care of the following points before starting the test.

1. Bring all the reagents and specimens to room temperature (20°C-30°C) before beginning the test. The immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.

2. Place the required number of HCV TRI-DOT test devices at the working area.
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.

4. 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.

5. If the solution does not soak-in within 40-60 seconds; observe the sample for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. and use a fresh device to re-run the test. Refer to "SPECIMEN / SAMPLE PROCESSING".

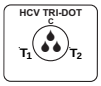
6. All solutions and sample should be added to the CENTRE OF MEMBRANE.

7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. **The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.**

13. TEST PROCEDURE

Step No. 1

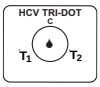
Add 3 drops of Buffer Solution to the centre of the device.



1

Step No. 2

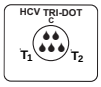
Hold the dropper vertically downwards and add 1 drop of patient's sample (50 µl serum or plasma) using the sample dropper provided. (use a separate sample dropper for each specimen to be tested).



2

Step No. 3

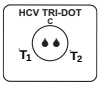
Add 5 drops of Buffer Solution.



3

Step No. 4

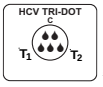
Add 2 drops of Protein- A Conjugate.



4

Step No. 5

Add 5 drops of Buffer Solution.



5

Step No. 6

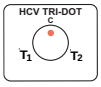
Read result immediately and discard the device immediately considering it to be potentially infectious.

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

14. INTERPRETATION OF RESULTS

NON REACTIVE RESULT

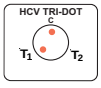
1. Appearance of only one dot at the control region **"C"** indicates that the sample is NON-REACTIVE for antibodies to HCV. (Fig:a)



a

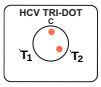
REACTIVE RESULT

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



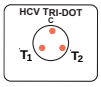
b

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



c

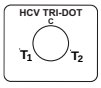
3. Appearance of all the three dots, one each at **"C" "T_1"**& **"T_2"** region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)



d

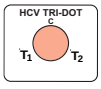
INVALID RESULT

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).



e

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).



f

IMPORTANT :

(i) Test dots "T_1" & "T_2" 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.

(iv) In case you have any problems in our HCV TRI-DOT, please call our Technical Customer Service Cell at Parwanoo, Himachal Pradesh, as per following details:
Ph: 0091-1792-232253

15. 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 assay 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 may show falsely reactive results.

(vii) The kit works best when used with fresh samples and when all the kit components are at room temperature (20-30°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) Rarely there may be an impression at the location T1&/or T2 where the antigens have been coated. These impressions will automatically get washed away & the membrane will be clear on addition of buffer solution in the first step of test procedure .However there will not be any impact on the test result.

(ix) 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.

16. PERFORMANCE CHARACTERISTICS

(i) The performance of **4th Generation** HCV TRI-DOT with reference to sensitivity and specificity has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1315 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples 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 + ve	HCV TRI-DOT - ve
40	ELISA +ve	40	-
1275	ELISA -ve	5	1270

Sensitivity : 100% **Specificity : 99.21%**

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - three HCV negative and seven HCV Positive (1 strong positive, 1 medium and 5 weak positive). The C.V. (%) of all the ten samples were within 10%.

17. 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.

18. REFERENCES

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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-08

Manufactured & Marketed By:

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