

# 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<sub>1</sub>" & "T<sub>2</sub>" 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).

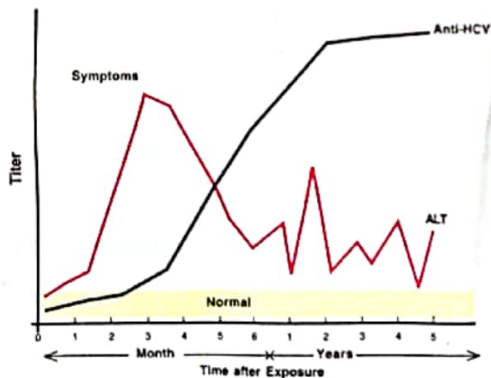


Fig.1 Hepatitis C Virus Infection  
Typical Serologic Course

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).

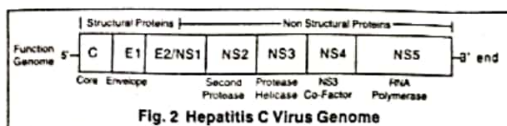


Fig. 2 Hepatitis C Virus Genome

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 immunofiltration membrane) includes two test dots "T<sub>1</sub>" & "T<sub>2</sub>" 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."

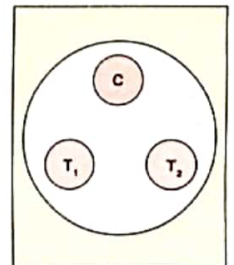


Fig. 3 Test Device

## 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).

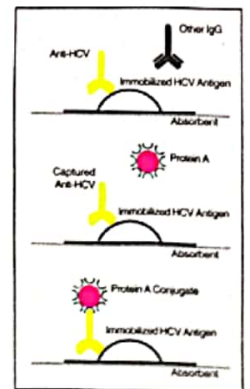


Fig. 4 Principle of the Assay