
Clinical Microbiology And Serology

INTRODUCTION

With advancement medical facilities in this 21st century, there is also an increase in providing a quality and affordable treatment. Infectious diseases is seen as an emerging global challenge for the world. Clinical Microbiology is the scientific study of pathogenic microorganism. It helps in diagnosis, management and treatment of infectious diseases. Clinical microbiology connects with other fields of medical science like molecular biology and serology to give information on microorganism.

Clinical samples such as blood and urine are collected from the patients for further tests. Fresh blood samples help us provide accurate information. Urine is now processed in microbiology lab and information is obtained from culture. Various agar media is used in clinical microbiology such as Blood Agar, Urichrome, MacKoney, Chocolate, MHE, XLD, and SDA. Streaking is to produce isolated provinces of a living being on an agar plate. This is valuable when we have to isolate life forms in a blended culture or when we have to ponder the state morphology of a life form.

Gram stains are used on clinical samples to differentiate between gram positive bacteria and gram negative bacteria. Serology is branch of medical science which deals with blood. And various serological tests are being conducted.

Some of them are:

1. HBSAG: This is a card test for diagnosis of Hepatitis B. it identifies the disease by identifying increase in amount of antibodies against the hepatitis B antigen in patient's blood serum.
2. VDRL: It is a test used to detect syphilis disease. TRUST(TOLUDINE RED UNHEATED SERUM) test kit is widely used to conduct VDRL test.
3. TYPHI: It is a test used to detect typhoid fever which is caused by bacterial infection by *S. paratyphi*, *S. typhi*, etc. This test is used to qualitatively detect rise is antibodies to lipopolysaccharides' on early encounters.
4. HCV: It is a rapid card test which provides qualitative, sensitive and visual diagnostic test for detection of antibodies against hepatitis C virus in human serum. This test is also called TRIDOT because it detects majorly 3 antibodies namely NS3, NS4, and NS5.

SAMPLE COLLECTION

1) BLOOD COLLECTION

1. First clean the arm(anticubital region) using 70% alcohol in anticlockwise direction.
2. Don't retouch the area, as it may contaminate the area
3. Now, break the paper on the Vacutainer Needle and inster it in the Vacutainer holder.
4. Apply tourniquet on the upper arm and inject the Vacutainer Needle. The median Cubital Vein to draw blood.

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5. Fill the tube 3/4 with blood according to anticoagulant concentration and the ratio mandated.
 6. Remove the needle and place cotton gauze on the site of injection to stop bleeding.
 7. Mix, the blood tube by making number 8 symbol in the air while holding the test tube.
 8. The blood sample is centrifuged to separate blood, plasma and serum.

2) URINE COLLECTION

Sample is collected in a sample container whose volume is 3-3.5 ml from the patient.

1) HEPACARD PRINCIPLE

Hepacard is a rapid and fast test to determine one of the most fatal disease known as Hepatitis B. This test is based on the antigen- antibody reaction. The hepacard is made up of substance that promote capillary action. The sample when poured in the sample well moves upward via capillary action and a colored line appears showing the test results due to antigen antibody binding on the surface.

REQUIREMENTS

- Hepacard
- Micropipette
- Serum sample
- Microtip
- Timer

PROCEDURE

- Clean the working area with 70% Ethanol.
- Bring all the test components at the room temperature i.e 20-30 C.
- Arrange all the reagents on the working desk as per their requirements.
- Bring the required number of hepacards and open the foil pouches.
- Now label the hepacards with patient's name or identification number.
- Add 2 drops (70 microlitres) of serum sample using the dropper provided.
- Observe the reaction for 20 minutes. Note down the result after 20 minutes.
- Discard the hepacard immediately.

APPLICATION

It is the rapid, simple and accurate method to detect early stages of Hepatitis B virus. HBV can be detected 2-5 weeks by HBsAg card test.

LIMITATIONS

HBsAg card test can be used in In Vitro diagnostics only. Only serum or plasma samples can be used in HBsAg card test. HBsAg card test is a qualitative test. It can only indicate HBsAg antigen on the surface HBV.

RESULT

Due to capillary action the sample moves upwards.

- 1) If the sample gives only one coloured line on the control region, then the test is said to be negative.
- 2) If there appears to be 2 coloured lines, both on the control region and test region then it is said to be positive.

2) HCV PRINCIPLE

The fourth generation HCV TRI-DOT is a rapid, visual, sensitive and qualitative method detecting antibodies against Hepatitis C Virus in human serum. The HCV TRI-DOT test is based on the principle of immobilization and absorption. The immunofiltration pad absorbs the serum sample and the reagents poured in it. It has been built in such a way that it has high ability to bind with NS3 antigens more effectively, efficiently and rapidly. HCV TRI-DOT CARD has markings upon it each pointing to different results such as 'C' = Control Region and 'T1' = T1 Antigen Region and 'T2' = Antigen Region.

REQUIREMENTS

- HCV TRI-DOT KIT
- Dropper
- Micropipette
- Gloves
- Lab coat

HCV TRI-DOT KIT COMPONENTS

S.no Ingredients Preparation

1. HCV tri-dot card Cut and open
2. Buffer solution Prepared
3. Protein A conjugate Prepared
4. Dropper Ready to use

PROCEDURE

- Clean the working area with 70% Ethanol.
- Bring all the test components at the room temperature i.e 20-30 C.
- Arrange all the reagents on the working desk as per their requirements.
- Open the HCV TRI-DOT KIT and label it with the patients name or identification number.
- Add 3 drops of HCV Buffer.
- Now add 1 drop of patient's serum (50 microlitres)
- Add 5 drops of HCV Buffer again.
- Add 2 drops of Protein A conjugate
- Add 5 drops of HCV Buffer.

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- Note down the result and discard the HCV TRI-DOT kit.

APPLICATION

HCV card test allows easy detection of HCV antigen produced in our body by the immune response. It gives a qualitative result. Early diagnosis of Hepatitis C virus.

LIMITATIONS

Fresh sample should be used. This is only a preliminary test and should be confirmed with tests too. It is a time bound test. Patient's with auto-immune disorders show positive result even if they are not infected by the HCV

RESULT

- When we obtain only 1 dot on 'Built in quality control' region C, then it is a Non-reactive for antibodies to HCV.
- When we obtain 2 dots, 1 at control region 'C' and 1 at test region 'T', then it is a Reactive for antibodies to HCV.
- When we do not obtain any dots after completion of experiment, then it indicates ERROR.