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DEVELOPMENT IN DIAGNOSTICS KIT AND DIAGNOSTIC METHODS FOR TESTING OF COVID19

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Novel Corona virus is a pandemic spreading worldwide , and it was first identified in china in Hubei province. Corona virus is a large group of viruses belongs to family coronaviridae. COVID 19 is a respiratory illness caused by a corona virus, also it is scientist's found that certain genes of SARS (sever acute respiratory syndrome) are similar, hence, later it was named by National Virology of institute as SARS Co-V-2. Day by day the virus spread is increasing meanwhile testing too. This article discusses the various diagnostic kits and methods used for detection of the COVID 19. Many test for corona virus disease are available now a days, and there are mainly four different test are widely used , they are 1. RT- PCR, 2. ANTIBODY TEST, 3.LAMP, 4. ELISA. Diagnostic methods have two kinds of testing for COVID 19 : Viral test and Antibody/Antigen

The FAD has granted Emergency use Authorizatio n for the collection of saliva

INTODUCTION: Molecular diagnosis techniques is a process of detecting and analysing disease or infection at molecular level i.e., at DNA , RNA & protein level. These techniques are under development for corona virus. The above mentioned four techniques are very easy for analysing as well as for researches. And the details diagnosis studies are given below:

RT-PCR (Reverse Transcriptase - Polymerase Chain Reaction) :

RT-PCR is a most commonly used in research and is a scientific technique use to identify the RNA viruses. Hence COVID 19 is a positive sense single stranded RNA virus. RT-PCR used to test for the presence of virus. It is more sensitive and quick,

results may obtain 3 to 5 hrs or a days. there are

various companies that **makes kits for RT-PCR, namely- GS COVID 19 RT digital PCR detection** Discussing about the **working of RT-PCR**, firstly sample should be collected into a viral transport medium from the Respiratory track - Nasopharynx or Oropharynzx . After the sample is collected chemicals like lysis buffer is added , ensuring the removal of proteins and other molecules and recruits RNA . Now using diagnostics kit provided, certain enzymes are added according to the instructions given in manual kit. Using PCR sample is amplified to more no. of copies, the enzyme Reverse transcriptase ensures the RNA to cDNA , and the **Fluorescent** marker get attached to the DNA which produce the light , based on intensity of

kit by Genosensor ; Gnomegen COVID 19 RT-PCR kit ; Logix smart corona virus RT-PCR test kit.

fluorescent light reaches to the certain threshold - declares the positive test or negative test.

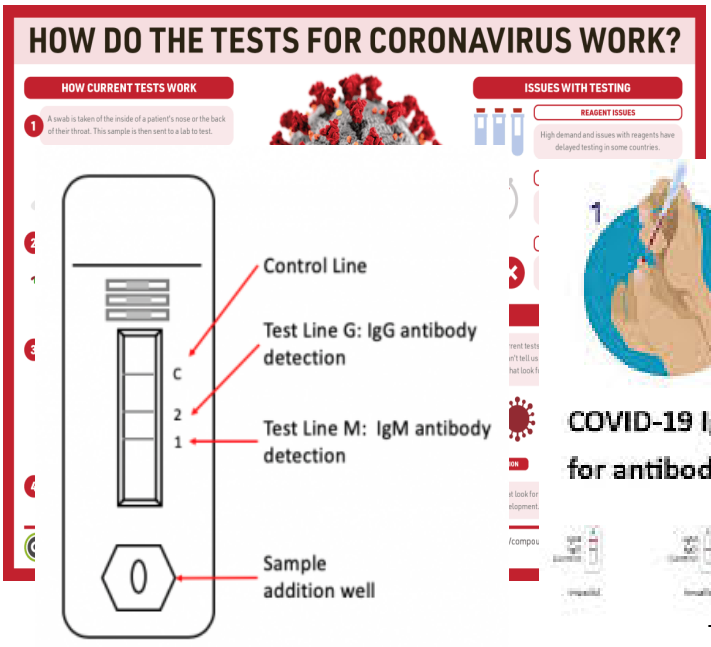
Antibody test can particularly detect the antibodies against the S protein of COVID 19

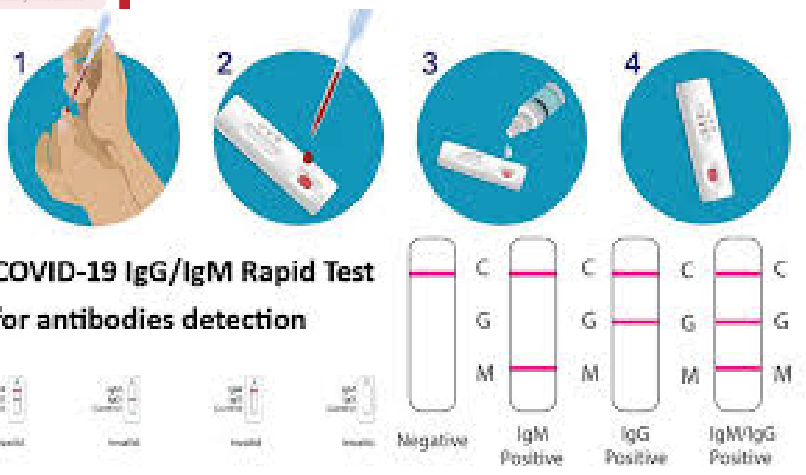
Results/report

Results may varies, in-case of positive test: the person is infected by the corona virus, where as in-case of negative test : the sample collected from the individual is not infected by the virus and virus is not present at time of collection or may the quality / quantity of the sample were poor

Serology test / Antibody test :

An antibody test is used to detect the presence of antibodies, which are specific proteins in response to viral infection, they can found in blood of an individual which shows





immune response to the infection. It is important for detection of infection whether previously infected or present infection.

- when any virus or a bacteria that infects human body, our body starts fighting against the infection, through the process our immune response produce two kinds of antibodies namely-

- IgM antibodies :develop early infection.
- IgG antibodies : develop later infection.

RT - LAMP uses DNA polymerase and 4-6 primes due to this LAMP is more specific than RT-PCR. And amplification

A blood sample is collected from a vein or a finger prick and the blood droplet is collected by a pipette and then placed onto a rapid diagnostic test device and few drops of buffer are added to ensure easy flow across the device. If the blood sample consist of antibodies that attached to the chemical which is present in device , this process occurs during the sample is allow to move through the device. The reaction between the chemicals and antibodies present in sample makes colour change with the control line, which result in formation of lines depending on antibodies present.

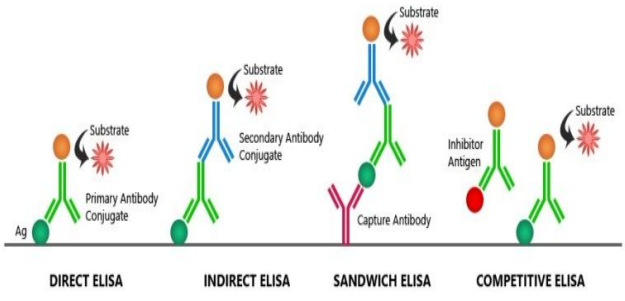
LAMP (Loop Mediated Isothermal Amplification) :

LAMP amplification, which is very similar to the RT-PCR. There are minor difference between RT-PCR & LAMP mentioned below:

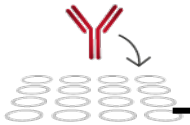
- Temperature is constant in case of LAMP (60 to 65).
- Rate of amplification of viral cDNA is more than compare to RT-PCR.

LAMP is a technically simpler can use easier for detection of virus. Working part of LAMP is very similar and can also sample can be collected from the mucus, if the patient contain coughing. Same like RT-PCR viral RNA is converted into cDNA which is then amplified further, the main advantage is that we can able to detect the presence of virus visually without the use of machine during the amplification process.

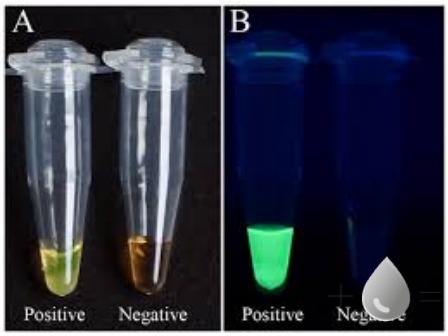
Will you ask how? Look at this - during the amplification process, reaction mixture itself turn's to cloudy precipitate, this is due to presence of "Magnesium pyrophosphate". The level of this chemical increases as the amplification rate increases, and this cloudy ppt can be seen with our naked eyes, this ensures the easy detection of presence of virus. Finally, for the accurate results provide by fluorescent dye using machine.



"LAMP cannot detect the inactive viral from an individual i.e., it can only detects the virus in an active state of an individual".

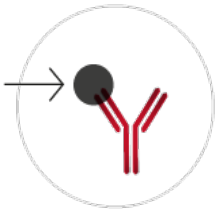


Quality control. This assay is worked based on enzyme-



Enzyme - Linked Immunosorbent Assay (ELISA) :

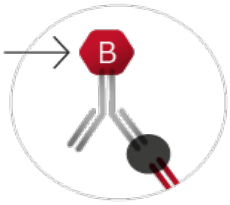
immunoassay to test antigens or antibodies & very specific. ELISA are of mainly four types, and they are shown in this picture given below:



ELISA is a biochemical assay used to detect antigens and antibodies. Used to check

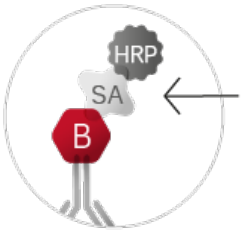
1. Antibody coating

Specific capture antibody is immobilized on high protein-binding plates by overnight incubation. Plates are blocked with irrelevant protein



2. Protein capture

Samples and standard dilutions are added to the wells and will be captured by the bound antibodies.

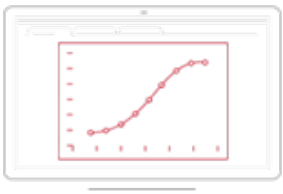


3. Detection antibody

Specific biotinylated detection antibody is added to the wells to enable detection of the captured protein

4. Enzyme conjugate

Enzyme conjugated with alkaline phosphatase or horseradish peroxidase is added to the wells and will bind to the biotinylated antibody.



5. Addition of substrate

Colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.

6. Analysis

Absorbance is measured in an ELISA reader and the amount of protein in the samples is determined.

RESULTS would depend on the change in colour after substrate is added. If colour changes this is a positive test for corona virus or antibodies present in sample collected. If there is no colour change observed that means no viral infection.

" ELISA test can able to detect the inactive virus from the sample i.e., this test can able to determine whether the sample collected from the individual is infected by virus in past, due to antibodies present in the sample"

DISCUSSION/CONCLUSION: These are the main four important diagnostic methods that can be tested for COVID 19 which are accepted by WHO for procurement, there are many companies working in the preparation of kits for rapid detection of virus infected by an individual. Each test are at different stages of development, validation and production. A combination of testing types used at different times may be useful for a patient management and population pandemic control of COVID 19.

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