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Perspective on the SARS-CoV-2 and Its Diagnostic Tests

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SARS-COV-19 AND COVID-19

Viral zoonotic infections such as H1NA and SARS are the recent health threat to humans with high transmission and fatality rate and cause a significant global burden. The first SARS epidemic hit the world in 2003 and affects more than 8400 individuals with approximately 800 deaths cases across 26 countries. It was assumed that the reemergence of this virus may cause a threat to humans.¹ Indeed, In December 2019, cluster of pneumonia cases caused by a novel coronavirus emerged from Wuhan, China. This pneumonia like disease is caused by novel coronavirus which is initially which is later named as SARS-CoV-2 based on the symptoms it produced in the infected individuals, severe acute respiratory syndrome (SARS). The disease was named officially as COVID-19 by World Health Organization (WHO) and Coronavirus Study Group (SCG) of International Committee proposed to name the novel coronavirus as SARS-CoV-2. Till date of this writing, SARS-CoV-2 infected more than 10 million individuals with mortality rate of 6.5% from every country in the world.

Coronaviruses belong to the family Coronaviridae and order Nidovirales. These are enveloped, single-stranded with a nucleocapsid, positive sense RNA viruses and characteristically have spike projections present on the membrane, which resembles to crown (Latin: corona) and hence termed as coronavirus.² These viruses have been reported to be able to infect humans and a wide range of animals including cattle, swine, chicken, cat, horse, camels, rodent, bats and snakes.^{3,4} In December 2019, a new coronavirus was identified as the cause of a disease outbreak that originated in China named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Diagnosis of COVID-19

Symptoms of COVID-19 typically appear between days 2 to 14 from the first exposure of SARS-CoV-2 and commonly noticed by observing fever, cough, fatigue, and shortness of breath. Since these are common to many viral infection, COVID-19 is confirmed by detection of SARS-CoV-2 nucleic acid by real time RT-PCR or antibody detection by serological methods in the throat swab samples collected from suspected patients.^{4,5} WHO recommends that samples pathological samples can be collected from respiratory tract such as nasal and oropharyngeal swabs, deep cough sputum, bronchoalveolar lavage, fluid pulmonary alveolus lavage fluid or blood of suspected patients.⁵⁻⁶

A. Diagnostic tests for the COVID-19: Detection of the virus

Real Time-Reverse transcription polymerase chain reaction (rRT-PCR)

rRT-PCR is the test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens. Since the COVID-19 virus only contains RNA, real time or conventional RT-PCR is used to detect it the most accurate laboratory methods for detecting, tracking and studying the COVID-19 coronavirus. It does this by capturing and amplifying regions of the virus' genetic material, usually the Spike protein, N protein or Envelope. To measure the viral RNA, it is converted to DNA (called 'reverse transcription'). They do this because only DNA can be copied or amplified which is a key part of the real time RT-PCR process for detecting viruses.⁷ Specific part of transcribed viral DNAs is got amplified many times using repeated temperature cycles in a PCR machine and then fluorescent markers are used to

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detect the virus. If the amount of fluorescence goes above a certain level, this confirms that the virus is present. The number of temperatures cycles the machine performs to reach this threshold is recorded to estimate how much virus was present in the patient sample. The lower the number of cycles, the more virus was present. An RT-PCR test is highly sensitive and reliable if performed on a sample from an infected part of the body whilst an active infection is occurring.⁸ However, RT-PCR involves specific equipment and very qualified specialists and takes upto 4–8 h to process and obtain final outcomes. This may add financial pressures and that may prevent effective deployment of these diagnostic methods.

Loop-mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is a process of nucleic acid amplification. It is rapid test that exhibits heightened sensitivity and specificity. It does not require expensive reagents or instruments and hence aids in cost reduction during this pandemic. Patients sample detection till date showed that 1–10 copies of viral RNA template per reaction are sufficient for successful detection. This is approximately 100-fold more sensitive than conventional RT-PCR test.⁹ Best chance for a rapid and robust assay for field diagnosis of COVID-19, without the requirement of specialized equipment and highly trained professionals to interpret results. Demerits of this test include not inclusion of an internal PCR inhibition control, necessitating duplication of reactions while testing, perceived complexity of the methodology, need of a complex primer design system which can constrain target site selection and resolution or specificity.¹⁰

B. Serological diagnostic tests for the COVID-19: Detection of the antibody

The detection of immunoglobulins such as IgG, IgM and IgA against the SARS-CoV-2 may play a complementary role to the RT-qPCR test in the diagnosis of COVID-19 and providing the immune status of individuals.

Lateral Flow / Colloidal Gold Immunochromatography

Antibody lateral flow immunoassays are typically a qualitative (positive or negative) test designed to detect antibodies - IgM or IgG alone or both together. Colloidal Gold is a qualitative membrane-based immunoassay for the detection of COVID-19 antibodies (IgG + IgM) in whole blood, serum or plasma. This indicates whether a subject has been exposed to the novel SARS-CoV-2 or not. This test consists of two test lines, an IgG line (anti-human IgG is coated in IgG test line region) and an IgM line (anti-human IgM is coated in IgM test line region). During testing, the specimen reacts with COVID-19 antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the

anti-human IgG or IgM in respective test line region.¹¹ If the specimen contains antibodies, generated by the individuals immune response to SARS-CoV-2, a colored line will appear in test line region. Antibody tests provide a hugely important ability to detect past infection with virus to identify asymptomatic people, people who have cleared the virus and so no longer risk being infected or spreading the virus to others. In addition, antibody tests are critical for assessing population spread of the virus and the level of 'herd' immunity in the population.¹²

Enzyme-Linked Immunosorbent Assay (ELISA)

Fully automated ELISA assays provide a semiquantitative in vitro determination of human antibodies of the immunoglobulin classes IgG and IgA against the SARS-CoV-2. ELISAs can be qualitative or quantitative and generally require a lab. These tests usually use whole blood, plasma, or serum samples. A plate is coated with a viral protein, such as a SARS-CoV-2 spike protein. Samples are incubated with the protein, allowing any antibodies to bind to it. The bound antibody-protein complex is the detectable following a wash with secondary antibodies attached with fluorescent or colour conjugates.¹² In the context of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM) develops in most patients within 7-10 days after symptoms of COVID-19 begin. IgG antibodies remain in the blood after an infection has passed. This antibody detection test may be useful to identify if the persons had COVID-19 in the recent past and that may protect you from future infection.

Neutralization assay

Virus neutralization is used for determining antibody efficacy and neutralization assays assessed whether sample antibodies prevent viral infection in test cells. This test can be employed using blood, plasma or serum samples. This assay can detect the functional neutralising antibodies that compete the virus to bind to the angiotensin-converting enzyme 2 host receptor and prevent the virus-host interaction and further infection.¹³ By varying antibody concentrations, researchers can visualize and quantify how many test antibodies block virus replication. A high-throughput assay to measure SARS-CoV-2 neutralizing antibodies is designed for COVID-19 serodiagnosis, convalescent plasma therapy, and vaccine development. This test can detect functional neutralising antibodies in an hour and differentiate them with binding antibodies.

Chemiluminescent immunoassay

Chemiluminescent immunoassays are quantitative lab tests. They sample blood, plasma, or serum. Samples are mixed with a known viral protein, buffer reagents and specific, enzyme-labelled antibodies. The result is luminescent. A chemiluminescent microparticle immunoassay

uses magnetic, protein-coated microparticles. Antibodies react to the viral protein, forming a complex. Secondary enzyme-labelled antibodies are added and bind to these complexes. The resulting chemical reaction produces light. The radiance is used to calculate the number of antibodies. This test can identify multiple types of antibodies, including IgG, IgM, and IgA.¹²

CONCLUSION

This signifies that different tests serve different purposes in the management of this COVID-19 global pandemic. While viral RNA testing enables point-of-care, acute detection using serological studies helps in contact tracing. It becomes mandatory to maintain systematic and coordinated efforts between the public, clinical, commercial and industry sectors to establish a diagnosis for COVID-19 to beat it.

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