



## Current Diagnostic Strategies for Covid-19

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Received: July 30, 2020

Published: September 16, 2020

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### Abstract

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first isolated from Wuhan from three patients with pneumonia. Afterwards the disease spread all over the world with high spreading rate. SARS-CoV-2 is closely related to the original SARS-CoV and it has all the features of the related coronaviruses in nature. As there is no vaccine provided yet, early diagnosis is very important for prompt management and to prevent the spread of highly contagious global pandemic. Currently nucleic acid amplification test, real-time reverse transcriptase PCR (RT-PCR) is used for diagnosis. Genes like E, N, S or ORF are targeted for screening or diagnosis. Serological assays are used for epidemiological studies and for identifying population at high risk for infection, while point of care molecular tests have the advantage of rapid, accurate and low cost which provide great help in diagnosis and quarantine of infected patients.

**Keywords:** Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2); Coronavirus Disease 2019 (COVID-19)

The novel Coronavirus disease 2019 (COVID-19) with estimated death rate of 5.7% has spread rapidly throughout the world [1]. Early diagnosis and quarantine of the patients are the methods to prevent the spreading of the disease. This has emphasized its laboratory diagnosis. "There will be no return to the old normal for the foreseeable future; but there is a road map to a situation where we can control the disease and get on with our lives; for this we focus on reducing mortality and suppressing transmission" said director general of WHO on 13 July. As per the latest data on 07 September 2020; WHO reported worldwide 27,032,617 confirmed cases and 881,464 deaths [2]. On same dates in India a total of 8,83,697 confirmed cases and 72,775 deaths had been reported [3]. This brings us to focus on the importance of cost effective, high sensitive and specific laboratory diagnosis to limit uncontrolled spreading as well as to appropriately treat those patients who have a serious infection.

The spread of this novel virus started as a small outbreak in Wuhan, China in December 2019, which has now evolved as pandemic

affecting individuals of all ages. Coronavirus disease is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) of coronavirusidae family (Betacoronavirus subgroup) [4]. Coronavirus has a single-stranded RNA. The viral envelope consists of a lipid bilayer, in which the membrane (M), envelope (E) and spike (S) protein and nucleocapsid (N) proteins are anchored [5]. Infection begins when the viral spike protein attaches to its complementary host cell receptor by membrane fusion. Spike protein interacts via angiotensin converting enzyme-2 (ACE-2) receptor. The binding affinity of SARSCoV-2 to ACE-2 receptors is about 10 to 20 times higher than SARS-CoV; so that SARS COV 2 has more severe infectivity than SARS-CoV and MERS-CoV. ACE-2 receptors are mainly found in, respiratory tract, cardiovascular, digestive, and urogenital systems. This causes the key pathophysiological complications leading to pulmonary and cardiovascular injury by COVID-19. It can also cause severe pneumonia, gastro-intestinal symptoms, acute respiratory distress syndrome, and acute kidney injury [6].

According to the current recommendations for laboratory diagnosis of COVID-19 from the Centers for disease control and prevention (CDC) the preferred testing method is the real-time reverse transcription-PCR (RT-PCR) test [7]. Nasopharyngeal or oropharyngeal swabs are recommended for the diagnosis of COVID -19 [8]. The diagnostic tests for COVID19 are classified in to two types; nucleic acid tests (Real-time PCR test) and serum test for IgM and IgG antibodies. Nucleic acid tests are for acute phase of the infection while serological tests detect patients who have developed antibodies.

For reliable and rapid detection of COVID-19, real-time polymerase chain reaction (RT-PCR) methods are employed. These methods convert RNA into complementary DNA by reverse transcription followed by amplification of DNA by using specific primers. RT-PCR method is significantly more sensitive for corona virus detection. The gene targets in RT-PCR method include open reading frame1ab, RNA-dependent RNA polymerase, nucleocapsid, spike protein and envelop genes. In the real time PCR assay two target genes were simultaneously amplified using specific primers [9]. In a positive RT-PCR test both genes are amplified. Even though it is the standard test it is time consuming and costly. Loop mediated isothermal amplification (LAMP) is a nucleic acid amplification technique, which has increased sensitivity and specificity and reduced cost per test. In LAMP technology the RNA is converted to DNA and further amplification by using multiple primers [10]. This increases the speed, sensitivity and amplification efficiency of LAMP when compared to RT-PCR [11]. The real-time reverse transcriptase-PCR (RT-PCR) is highly specific and simple quantitative assay for the detection of SARS-CoV-2. It also has the required specificity for early diagnosis of COVID-19 [12].

Incubation period of SARS COV-2 is with a range of 4-14 days. Specific IgM and IgG antibodies start to become detectable 4-5 days of infection. Specific IgM antibodies are detectable within 8-14 days and IgG will be detectable after several weeks [13]. There are combined tests for both antibodies and may take 14 days after infection to get positive result. Serological tests are useful for diagnosis and also for epidemiological surveillance of the disease. Lateral flow immunoassays, ELISA and chemiluminescence assays are the techniques used rapid screening of patients for antibody detection against COVID-19 in serum [14]. Nowadays monoclonal antibodies are used for the detection of viral antigens in epidemiological studies. Nonspecific markers like lactate dehydrogenase, alanine transaminase, C-reactive protein are elevated following COVID-19

infection. Lymphopenia and increased levels of interleukin-2 and interleukin-7 are seen in patients. Effective use of molecular genetic tests and serological assays in combination is the need of the hour to improve diagnostic accuracy

## Conclusion

To conclude the diagnosis of SARS COV2 can be done by Real-time PCR test which is the reference standard for confirmatory test while, viral nucleic acid sequencing to detect the homogeneity to SARS-CoV-2 or Serum test for SARS-CoV-2 specific IgM and IgG antibodies and chest imaging can also be considered.

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