# THE ROLE OF LABORATORY ANALYSIS IN IMPROVING THE EARLY DETECTION, DIAGNOSIS, TREATMENT AND MONITORING OF COVID-19 (LITERATURE REVIEW)

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***Abstract.*** *In this article, we review the latest laboratory diagnostic technologies and methods for SARS-CoV-2 and consider ways to improve the disease monitoring system for developing preventive measures aimed at preventing the disease and managing the outbreak.*

***Keywords:*** *COVID-19, reverse transcription-polymerase chain reaction (rRT-PCR), droplet digital polymerase chain reaction (ddPCR), serological testing, antigen testing, monitoring system.*

## Introduction

The global pandemic of the 2019 coronavirus disease (COVID-19) has caused a serious health crisis in all countries. More and more tests are needed to fight the virus and prevent or slow its spread. To date, there is a need for more sensitive, specific and convenient methods of detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The organization and conduct of these expanded tests will allow for the clinical diagnosis of COVID-19, the diagnosis and the selection of the correct treatment tactics, as well as the development of specific preventive measures. In this article, we review the latest laboratory diagnostic technologies and methods for SARS-CoV-2 and consider ways to improve disease surveillance.

The global pandemic of COVID-19 has threatened the lives of millions of people since its outbreak in 2019 [1,2,3]. This disease has a negative impact on human health, economic growth, social stability, and the civilization process of human society. Therefore, urgent measures are being developed by all countries to detect, prevent, monitor, and manage this epidemic in general [4,5].

Although there is still no vaccine that can provide absolute protection, the development of rapid and reliable diagnostic methods to diagnose symptomatic or asymptomatic cases of COVID-19 is of great importance [6]. Rapid and reliable diagnosis is key to rapid and reliable treatment decisions and development of appropriate quarantine strategies [7,8].

Although reverse transcription-polymerase chain reaction (rRT-PCR) is currently considered the most popular laboratory test for the detection of COVID-19 infection, chest X-ray, computed tomography (CT), [9,10] portable chest X-ray [11], and some traditional test methods such as flexible bronchoscopy [12] are also being used as adjunctive tools [13]. However, from low viral load samples, advanced technologies (ddPCR, LAMP, RPA, CRISPR-Cas)[14,15,16] and nanotechnology-based biosensors [17] and artificial intelligence based big data analysis are limited [ 18].

Therefore, the development of advanced, rapid, and timely diagnostic methods is a necessary complement to overcome the limitations of conventional methods and greatly enhance our chances of defeating the epidemic.

Although this issue is an important topic, it focuses on different areas, such as nucleic acids, serological tests, new materials or artificial intelligence. In this work, instead of repeating all the details presented in previous publications, we highlight the shortcomings of the current diagnostic options and suggest potential solutions. we aim to provide a comprehensive description of available detection methods from a laboratory perspective. Here, we review and summarize several COVID-19 detection technologies, as well as their advantages and disadvantages.

**Real-time reverse transcription-polymerase chain reaction (rRT-PCR)**

The rRT-PCR method is recommended by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) for the detection of SARS-CoV-2 [19, 20]. The positive detection rate of rRT-PCR assays after the onset of symptoms is 0 It has been reported to reach 89% within 4 days and to 54% after 10-14 days [21]. Symptoms of viral infection may begin 5-6 days before the onset of symptoms and may last up to 37 days in survivors [22,23], longer in some patients with certain chronic conditions, including malignancies, due to weakened immune systems [24,25, 53]. Patients with a low viral load are difficult to diagnose with rRT-PCR assays in the early and convalescent stages of COVID-19 [26,27]. As a result, false-negative results prevent the timely application of correct treatment tactics to the disease, and as a result, the general condition of the patient worsens. It is recommended that more sensitive methods should be used in such cases [21, 28].

**Droplet digital polymerase chain reaction (ddPCR)**

The droplet digital PCR (ddPCR) method is a novel approach for absolute target nucleic acid quantification without the need for a standard curve. Using the same primers and probes as rRT-PCR, ddPCR provides improved sensitivity and specificity for low viral load detection [29,30]. Each microdroplet contains zero or one copy of the target fragment composed of thousands of micro PCR reactors. Based on Poisson statistics, the number of DNA molecules in the original sample was directly calculated, which reduces the subjectivity of the analysis by determining the signal threshold and eliminating the need for standard curves [ 30 , 31 ].

Although it requires skilled technicians and specialized equipment, ddPCR is an ideal method for medical management of COVID-19. The method helps identify new low-virulence cases and quarantine close and general contacts at an early stage, thereby helping to prevent human-to-human transmission in time. In addition, changes in viral copy number provide evidence for assessing treatment efficacy and viral clearance rates and for continuous monitoring of viral load in convalescent patients, which may inform policy development for the management of isolated patients.

**Serological examination**

Due to asymptomatic infections or limited detection [32, 33], not all patients with COVID-19 receive direct evidence of infection. Therefore, serological tests based on the detection of specific SARS-CoV-2 antibodies are an important tool for ancillary purposes [34, 35]. In addition, due to the dynamic change of the level of antibodies against SARS-CoV-2 in different periods of infection, the detection of serological antibodies plays a major role in the detection of previous infection, convalescent diagnosis, epidemiological investigation and evaluation of the effectiveness of the vaccine.

Understanding specific antibody profiles is critical for identifying COVID-19, predicting disease severity, and assessing long-term immune function. Some studies have shown that the average time of seroconversion for IgM was 10-12 days after the onset of symptoms, and in some

patients it could be detected within 1 week and increased and reached a peak at 2-3 weeks, then began to decline significantly after 4-5 weeks [35,36].

**Antigen test**

Antigen detection refers to the detection of parts of SARS-CoV-2 viral surface proteins, which helps in the early diagnosis of SARS-CoV-2 infection [37]. The main structural proteins of SARS-CoV-2 include nucleocapsid protein (N), spike protein (S), envelope protein (E) and membrane protein (M)[38, 39]. Detection of SARS-CoV-2 protein in different types of samples can help to rapidly classify patients with susceptibility to COVID-19 infection and has the advantage of reducing processing time and reducing costs.

Antigen detection is usually highly specific but generally not as sensitive as nucleic acid detection [40]. The sensitivity of the antigen test is more reliable when the viral load of nasopharyngeal or oropharyngeal swab samples is high, and it is better to use this test mainly during the first week of SARS-CoV-2 infection [41, 42]. Therefore, it cannot be used as the sole basis for the diagnosis of COVID-19. At the same time, due to its low cost, rapid results, and wide deployment, antigen detection can be used in auxiliary screening of suspected patients, screening of asymptomatic high-risk groups, and routine surveillance, especially in high epidemic situations.

Although direct evidence reflecting SARS-CoV-2 infection is etiological evidence, the presence of false-negative and false-positive results may lead to mismanagement [43,44]. Therefore, it is important to use non-pathogen-based laboratory results in the screening, diagnosis and differential diagnosis of COVID-19. Such findings help predict disease progression and guide treatment decisions, especially when etiological evidence is negative [45, 46].

Detecting the disease in its early stages and stopping its spread through comprehensive screening, rapid identification, and isolation of all infected individuals are key steps to stop the epidemic [47, 48]. The current rapid global spread of COVID-19 poses a challenge in the allocation of medical resources, and laboratory-based identification methods can facilitate aggressive screening, early diagnosis, and effective prevention to minimize the risk of transmission [49,50].

**Prediction of disease progression**

Laboratory analysis results that help to identify patients at risk of developing a serious disease early and correctly, help to improve the patient's condition and rationally allocate medical resources. Many studies have shown that monitoring the immune response of patients with COVID-19, including the detection of cytokines, chemokines, and lymphocyte subsets, can be one of the bases for predicting the progression of patients to severe conditions [51,52].

Comprehensive analysis of laboratory results also helps in treatment decisions and monitoring and early detection of possible complications. These results show that laboratory findings can reflect the immune status, disease progression, organism damage and treatment process, and can be rationally interpreted from them. use may provide more evidence for early screening and diagnosis to predict disease progression and create individualized treatments.

**Conclusions and perspectives**

- During the current pandemic and future epidemics, laboratory testing remains the cornerstone of public health surveillance and mitigation strategies. It provides the necessary guidance for the continuous improvement of detection methods, prevention, treatment and vaccine development. Currently, pathogen-based laboratory findings are the most commonly used direct evidence to determine whether patients have SARS-CoV-2 infection.

- It can be used as indirect evidence to detect easier-to-administer antibodies, assess SARS-CoV-2 infection, evaluate vaccine efficacy, and reflect the current infection status of patients. At the same time, the development of the disease can be comprehensively evaluated depending on the type and titer of antibodies. Nucleic acid and antibody detection at different stages of infection had different sensitivity values, especially in the middle and later stages of infection, because nucleic acid detection rate decreased and antibody detection rate increased. Co-detection of nucleic acid and antibody can reduce the rate of missed diagnosis.

- Samples suitable for antigen detection are usually samples from the infected site, mainly nasopharyngeal swabs and bronchoalveolar lavage fluid. The quality of the sample, the site of infection, and the amount of virus expression greatly affect the detection results. Currently, further testing and preparation of antibodies, mainly nasopharyngeal swabs and bronchoalveolar lavage fluid, with high affinity and specificity are needed to develop antigen detection reagents.

- In general, detection methods targeting nucleic acids, antigens or antibodies always play an important role. We recommend that future research efforts focus on improving testing capabilities, simplifying the testing process, and providing faster results in a user-friendly format.

The COVID-19 pandemic has brought laboratory analysis processes to a new level, with the addition of big data, artificial intelligence and other technologies, as a result of which they are actively helping to form a unified patient database. At the same time, the digitization of the disease monitoring system provides an opportunity to analyze and evaluate all updated data, to provide expanded suggestions for making decisions on diagnosis and treatment. Improving this system will help to develop the most effective methods to meet the current needs for monitoring specific and cost-effective diagnostics of COVID-19, accurate diagnosis and treatment of the disease, promotion of data processing in developing countries with limited technical infrastructure.

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