

NEWS AND VIEWS

Diagnostic tests for Coronavirus Disease 2019. What happens behind the assays?

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DOI. 10.21931/RB/2020.05.02.16

Abstract: Pandemic caused by Coronavirus Disease 2019 (COVID-19) shows a plethora of clinical manifestations from the absence of symptoms to the development of pneumonia and even death. Nowadays, the number of new infections estimated to stem from a single COVID-19 case is between 2 and 3. For this reason, a rapid diagnosis will allow the massive screening of the population and the isolation of carriers and asymptomatic people. However, selecting an appropriate diagnostic test might be highly relevant, depending on the prevalence of the illness and the population to be tested. This communication has as purpose to describe the methodological tests employed to the COVID-19 diagnosis and analyze the pros and cons of them.

KeyWords: COVID-19, diagnosis, immunoassays, q-RT-PCR.

COVID-19 Diagnostic Tests

Coronavirus Disease 2019 (COVID-19) is caused by a novel coronavirus, which is closely related to two bat-derived severe acute respiratory syndromes (SARS)-like coronaviruses¹. Patients may undergo different manifestations from asymptomatic carriers to developing interstitial pneumonia, multi-organ failure, and death². This illness emerged in December 2019 in Wuhan, China, from where it spread worldwide (Singhal 2020), infecting over 4 000 000 people and causing the death of around 290 000 people³.

Hitherto, many research groups are working to develop an accurate diagnostic method. In particular, they have focused on two principal approaches: (i) molecular and (ii) immunoassays, which aim to be the primary diagnostic alternatives in both developed and developing countries⁴ (Figure 1).

Regarding molecular analysis, approaches based on quantitative Reverse Transcription Polymerase Chain Reaction (q-RT-PCR) have been designed. Some q-RT-PCR protocols analyze two genes. The amplification of one gene is interpreted as a positive screening, while the presence of the second one is interpreted as a confirmatory result. On the other hand,

other methodologies examine three or more genes, and the test is interpreted as positive only when the three genes are detected⁵.

The United States Centers for Disease Control (CDC) q-RT-PCR detects specific viral SARS-CoV-2 genes of the viral nucleocapsid (N1 and N2) while the methodology of the World Health Organization (WHO) targets the SARS-CoV-2-RNA-dependant RNA polymerase (RdRP) and envelope (E) genes. Both of them use a cycle threshold of less than 40 as the criterion for positivity^{6,7}.

According to the Guidelines of the Korean Society for Laboratory Medicine and the Korea Centers for Disease Prevention and Control the q-RT-PCR must be carried out to (i) confirm patients' release from quarantine, (ii) screen asymptomatic people related to COVID-19 patients and (iii) make a differential diagnosis among COVID-19 and other respiratory syndromes⁵.

Although q-RT-PCR is considered as the confirmatory diagnostic test, the principal disadvantage of this method is the high number of false-negative results. The causes of this

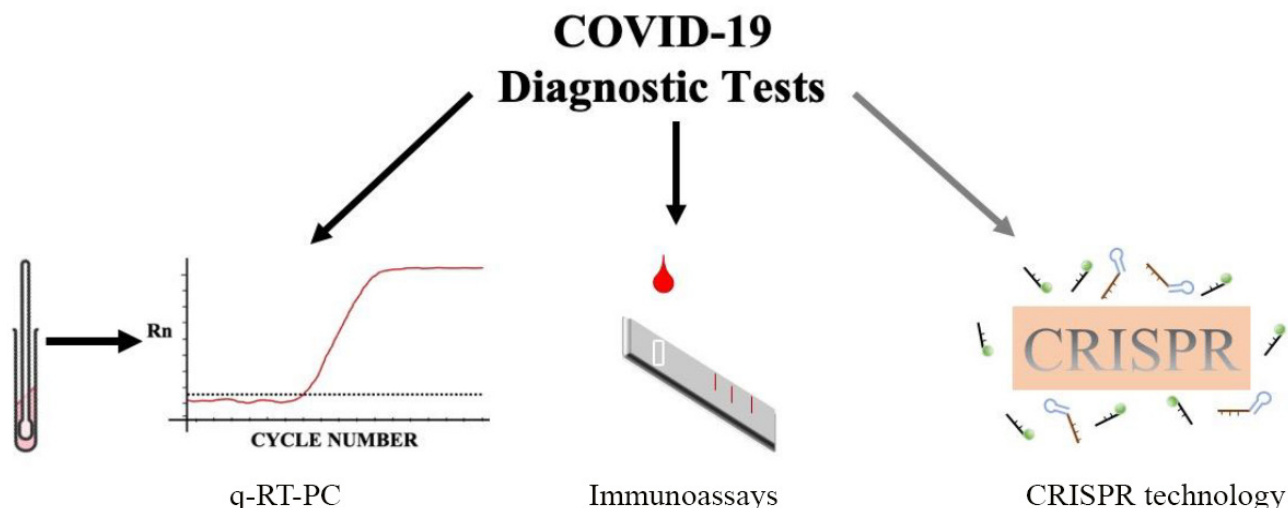


Figure 1. COVID-19 diagnostic tests. Quantitative Reverse Transcription Polymerase Chain Reaction (q-RT-PCR) is considered the gold standard to diagnose COVID-19. Several immunoassays have been developed; some of them are based on the lateral flow principle these tests is being used as rapid screening tests. Nowadays, Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR) based tests are emerging as a recent alternative, although they have not been certified as an in vitro diagnostic test yet. For more details, see the text.

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inconvenience might be: (i) poor specimen quality, (ii) improper samples handling or transported, (iii) a viral genetic mutation, (iv) presence of PCR inhibitors, or even (v) samples with low viral loads⁵.

On the other hand, considering the immunogenic response of S and nucleocapsid viral proteins that trigger immunological response associated to immunoglobulins production from 17 and 23 days after disease onset⁸, with IgM and IgG seroconversion within 20 days after symptoms⁹ different immunological approaches based on lateral flow, ELISA, and chemiluminescence have been developed as diagnostic immunoassays¹⁰.

Previous studies describe diverse results on the sensitivity and specificity of lateral flow tests. For example, Li *et al.* (2020) analyzed the accuracy of this serologic test in 397 SARS-CoV-2 patients and 128 healthy people confirmed by q-RT-PCR. The results showed that the sensitivity and specificity values for the immunoassay were 88.66% and 90.63%, respectively¹¹. These results were similar to those reported by Castro *et al.* (2020). These researchers, in a meta-analysis carried out in Brazil that had a purpose of setting the accuracy of available lateral flow tests to diagnose COVID-19 in that country, found sensitivity values between 55% and 100% and specificity between 94% and 100%¹².

However, the applicability of these tests depends on the prevalence of the disease. In a high-prevalence location with more than 300 COVID-19 cases among 12000 inhabitants, 49 patients were randomly selected and were evaluated using a lateral flow immunoassay IgM/IgG vs. the q-RT-PCR. The results showed only 8 q-RT-PCR positive tests were positive to the immunoassay (sensitivity: 36.4%), and from 27 q-RT-PCR negative samples, 24 were detected as negative by the immunoassay (specificity: 88.9%)¹³.

Other immunological methodologies, such as ELISA and chemiluminescence, have similar accuracy to the lateral flow immunoassays. For example, Adams *et al.* (2020) reported the SARS-CoV-2 IgM/IgG ELISA sensitivity and specificity values of 85% and 100%, respectively¹⁴. Alike, IgM/IgG titers measured among 43 COVID-19 patients and 33 health people by chemiluminescence showed a sensitivity of 48.1% and 88.9% and specificity of 100% and 90% for each immunoglobulin, respectively¹⁵.

Another alternative to COVID-19 diagnosis might be CRISPR (Cluster Regularly Interspaced Short Palindromic Repeats) technology. Gootenberg *et al.* (2017) previously reported the SHERLOCK system (Specific High -Sensitivity Enzymatic Reporter UnLOCKing) as a virus CRISPR-based diagnostic platform which takes advantage of the unspecific catalytic activity of the Cas13a enzyme releasing a fluorescent RNA reporter previous an isothermal amplification¹⁶. This system has been improved recently by a lateral readout platform, which guarantees a quantitative and rapid detection of specific nucleic acids¹⁷.

Until now SHERLOCK system has not been tested on biological samples. However, a modification of this platform termed DETECTR (DNA endonuclease-targeted CRISPR trans reporter)¹⁸ was probed on samples, although its use in diagnosis has not yet been approved by the U.S. Food and Drug Administration. Recently, Broughton and co-workers (2019) described a modification of this system based on CRISPR-Cas12 lateral flow assay as a visual and faster alternative to diagnose COVID-19¹⁹.

Conclusions

Immunoassays might be an alternative for the rapid diagnosis of COVID-19 as a complement to viral nucleic acid detection specially among carriers, asymptomatic, symptomatic patients and health sector workers²⁰. However, one possible disadvantage of the immunoassays is the fallen of the IgG titers at 8 weeks post symptoms onset, although these titers remain above the detection threshold¹⁴ being possible to detect anti-SARS-CoV-2 IgG up to 50 days from symptoms onset^{21,22}. On the other hand, previous studies on 11 patients diagnosed with pneumonia due to coronavirus at day 240 after symptoms onset showed that all patients were still positive to SARS-CoV anti-nucleocapsid IgG²³. SARS-CoV-2 could have different IgG kinetics than anti-nucleocapsid IgG of SARS-CoV.

Considering the highly variable performance of lateral flow immunoassay devices¹⁴ it is urgently needed to address studies to analyze the diagnostic yields of the immunoassays for COVID-19 diagnosis. Against this background, new molecular technologies based on editing gene tools might be a feasible, cheap, and rapid alternative to the existing COVID-19 diagnostic systems¹⁹.

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Received: 9 May 2020

Accepted: 14 May 2020