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Molecular and serological tests for COVID-19

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Abstract:

The outbreak of coronavirus disease 2019 (COVID-19) has become a global public health problem. There is little known for test utilization and diagnosis. Tests for COVID-19 include molecular tests and serological tests. Nucleic acid testing is a gold standard, while serological tests are used in seroprevalence. The tests should be selected according to the time course of the virus and serological response. The targeted genes are several. In our country, real-time polymerase chain reaction kit targeting RNA-dependent RNA polymerase gene fragment is being used. Common sample types are nasopharynx and/or oropharynx swabs. Patients with pneumonia sputum, bronchoalveolar lavage fluid, etc., should be tested. The peak concentrations of viral load reach before day 5, and the virus can be detected until the end of the 1st week after the onset of illness from nasal-pharynx. There are also rapid tests either detect the viral components in nasopharyngeal secretions or antibodies. IgM/IgA and IgG antibody are detectable on day 5 and day 14, respectively, after symptom onset. Enzyme-linked immunosorbent assay kits based on recombinant nucleocapsid protein and spike protein are expected to give more reliable results. Rapid diagnostic tests detecting neither antigen nor antibody is the first choice of the World Health Organization for diagnosis but is recommended to be used for surveillance. Ideal guidance in current circumstances is to confirm cases with available tests by following national recommendations so that we should take action as soon as possible, give appropriate therapy, and determine their contacts for infection prevention.

Keywords:

Coronavirus disease 2019, molecular tests, polymerase chain reaction, rapid test, serological tests

Introduction

There are several coronaviruses that can cause infections in humans. While the endemic human coronaviruses HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43 cause mild respiratory disease, the zoonotic Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus (SARS-CoV) have a higher case fatality rate. In December 2019, a group of patients with a novel coronavirus was identified in Wuhan, China.[1] In the beginning, the virus was named 2019 novel coronavirus, later International Committee of Taxonomy of Viruses has named it as SARS-CoV-2.[2] The World Health Organization (WHO) tagged it as COVID-19 virus in its current documents, and it is the causative of coronavirus disease 2019 (COVID-19).

The outbreak of COVID-19 has become a global public health problem. Since there is no specific treatment or vaccine yet, early diagnosis and sufficient isolation period for infected individuals is of primary importance. Clinical, laboratory, and radiological features are used to diagnose. For the diagnosis of COVID-19 symptoms and radiological findings are nonspecific. But to confirm the SARS-CoV-2 infection

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amplifying a specific genetic sequence in the virus by nucleic acid-based polymerase chain reaction (PCR) can be used. The WHO published interim guidance for laboratory testing for COVID-19 suspected human cases on March 19, 2020 (WHO 2020).[3]

As SARS-CoV-2 is a new virus, what we know is little about optimizing clinical outcomes and using available tests to manage diagnosis. We are confronted with new questions and need their answers to cope with this situation. To begin with guiding the use of testing, certain points need to be taken into account; what are the available tests and what is the appropriate time to use these tests and for whom to test? For the diagnosis of SARS-CoV-2, there are two main test approaches: to detect the virus or to detect the host's response to the virus. In addition, it is important to select the tests according to the purpose and either the value or the benefit of the test, whether it is beneficiary to the individual or to the public. In this context, the selection of the test depends on several criteria and several goals[4] [Figure 1].

While having no proven effective therapy or vaccine for that unprecedented pandemic caused by a new virus, the diagnostic testing we have becomes a crucial tool. The stage of COVID-19 disease determines the test to be selected. The accuracy of the test is affected not only by the time of sample collection but also by sample quality, especially in molecular tests. Although SARS-CoV 2 is a new virus, depending on the knowledge so far, the host response and the characteristics of the virus in terms of the time course of the virus and serological response of the host is illustrated below [Figure 2].

The tests used in the diagnosis of the disease, as in all viral diseases; is based on the representation of the virus itself, its genetic material or antigenic material, or the antibodies (Ab) against the virus. Although virus culture can be done to show the virus itself, it carries risks for laboratories and requires specialized equipment, so it is not routinely applied in clinical laboratories. In clinical diagnostic laboratories, molecular tests, in which the genetic material of the virus is detected, and serological tests, in which antigens or Ab against the virus are detected are used.

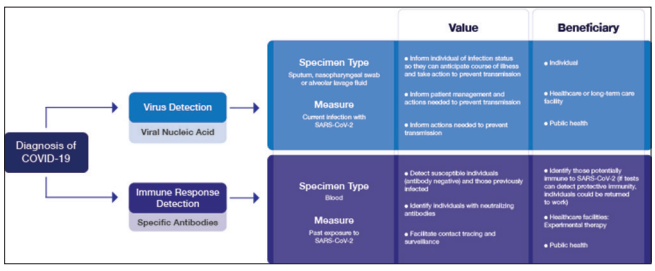

Figure 1: Laboratory diagnosis of coronavirus disease 2019

Tests

Molecular tests

The tests in use for the diagnosis of COVID-19 include the detection of viruses by genomic techniques using either PCR-based method or deep sequencing.[5-7] A few weeks after the first cases diagnosed in Wuhan, China, they published the full genome of the novel coronavirus was on January 10, 2020. And is followed by a group of scientists who first performed a PCR-based diagnostic protocol for COVID-19 using nose and throat swab sample from a patient. Hence, this protocol has been selected by the WHO is in the current usage. By using the genetic similarities between SARS-CoV-2 and its close relative SARS, an analysis was conducted. Later, that analysis was refined using the SARS-CoV-2 genome data to target the unique viral genes of the newly discovered virus. The test detects the presence of SARS-CoV-2's E gene, which codes for the envelope that surrounds the viral shell, and the gene for the enzyme RNA-dependent RNA polymerase (RdRp).[3] There is not only one protocol in use, except from the WHO's recommendations, The US Centers for Disease Control and Prevention, has developed a different assay seeking for three sequences in the N gene, which codes for the nucleocapsid phosphoprotein found in the virus's shell, also known as the capsid. The assay also contains primers for the RdRp gene. It is also a PCR-based testing protocol but only targeting variable genetic material of the virus.[8] (ECDC) European Centre for Disease Prevention and Control encourages the timely sharing of sequence data generated from a representative sample of positive specimens. The publically available sequence database GISAID accepts the upload of SARS-CoV-2 sequences. The next strain offers genomic evolution analysis and phylogenetic visualization of SARS-CoV-2.

Routine confirmation of COVID-19 cases is done by real-time reverse transcription PCR (rRT-PCR) and

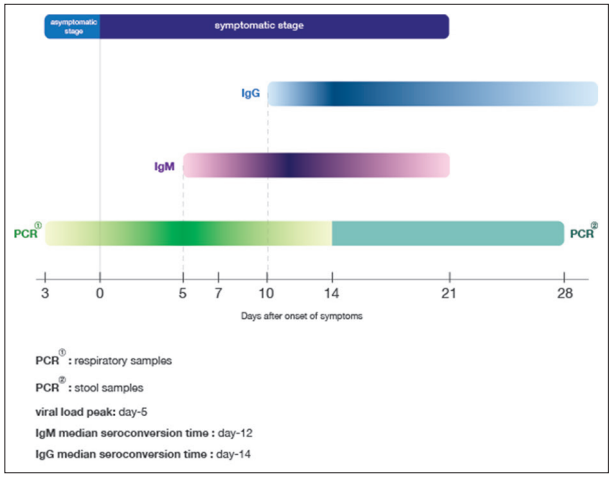

Figure 2: Molecular and serological response

nucleic acid sequencing when necessary.[3] Although different protocols have been published targeting the N, E, and S genes for molecular tests, it is sufficient to adopt a simpler algorithm, such as scanning with a single descriptive targeted rRT-PCR, where the SARS-CoV-2 virus is commonly seen. With this approach, in our country, tests are carried out with one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) kit targeting the RdRp gene fragment at the centers authorized by the Turkish Ministry of Health, General Directorate of Public Health Microbiology Reference Laboratory.[9] The RdRp gene-targeted Wuhan-RdRp oligonucleotide set gives only positive results with SARS-CoV-2. The kit's limit of detection has been determined as 5.6 copies SARS-CoV-2/reaction, and its analytical sensitivity is 99.4% and specificity is 99.0%.

Although changes have been made in patient and contact management algorithms over time, the main point is from where and when to take samples for viral RNA detection. The viral RNA in the collected sample determines the performance of the tests that detect viral RNA. Nasopharynx and/or oropharynx swabs are commonly tested samples.[10] It is preferred to combine both oral and nasal samples from a patient and test simultaneously in a single reaction. After collection, swabs are placed into a viral transport medium in which can be stored till the testing time. In routine practice, one or more negative results, especially with a nasopharyngeal and/or oropharyngeal swab, do not exclude the possibility of COVID-19 virus infection. False-negativity of PCR results has to be taken into account. These reasons may be due to the preanalytical phase, such as poor quality of the specimen, the time clinical specimen collection, the inappropriate shipment of the sample or technical reasons or, more importantly, there may be insufficient viral material in the specimen.

For patients with pneumonia, sputum, and bronchoalveolar lavage (BAL) fluid are the most-preferred lower respiratory tract specimens to test. The detection rates are variable for each sample type during the illness period. Wang *et al*. searched for SARS-CoV-2 RNA in a group of 205 patients with COVID-19 and tested 1070 different specimens. The positivity rates of different clinical specimens they found are as follows; BAL 93%, sputum 72%, nasal swabs 63%, pharyngeal swabs 32%, feces 29%, and blood 1%.[11] In numerous studies, nasal or oropharyngeal samples from patients with pneumonia detected negative by PCR while they were positive for lower respiratory tract specimens.[12-14] The characteristic radiological findings of COVID-19 pneumonia were seen on these patients, and their PCR test results were either negative or weakly positive at first. In these cases, repeated testing is suggested because as time passes, until the end of the 1st week after the onset of illness, the possibility of detecting the virus in the nasal-pharynx increases. Diagnostic tests show that simple throat swabs will provide sufficient sensitivity when symptoms are still mild or in the prodromal infection stage. A study shows that peak concentrations of viral load were reached before day 5, and successful live virus isolation from throat swabs can be done. Altogether, this indicates active virus replication in the upper respiratory tract tissues. Also, SARS-CoV-2 uses angiotensin-converting enzyme 2 as a receptor and have a quite similar excretion kinetic in sputum with active replication in the lung. The symptoms mostly decrease by the end of the 1st week, but until the second week, viral RNA is still detectable in the throat swabs. Stool and sputum samples remained RNA-positive over three weeks in six of the nine patients, although the symptoms resolved completely. The prolonged viral shedding in sputum has to be taken into account for infection control and discharge management.[15]

As evidence of active replication in the gastrointestinal tract, several studies showed the prolonged presence of SARS-CoV-2 viral RNA in fecal samples. Fecal samples of 41 (55%) patients out of 74 were positive for SARS-CoV-2 RNA, while respiratory samples of this group show positivity persistence for a mean of 16.7 days and fecal samples for a mean of 27.9 days after symptom onset.[16] Case reports showing positive fecal, but negative oropharyngeal tests also exist.[17]

Researchers studied viral load in different clinical material and what they found is the similarity of viral load both in an asymptomatic patient and symptomatic patients. That studies suggest that the asymptomatic or minimally symptomatic patients may have a role in transmission[10] while another study points out that patients with high viral loads show severe clinical outcomes.[18] The viral loads in throat swab and sputum samples peaked at around 5–6 days after the beginning of symptoms, ranging from around 104 to 107 copies/mL during this period.[19] During the pandemic, there is no point in measuring viral load in routine practice. But to show the relation between the SARS-CoV-2 viral load and the disease severity and prognosis, more studies are needed.

There is an increased requirement for test kits in this time of pandemic, and companies are working hard on meeting this need. Most are applying the same real-time PCR methods already in use, while others are working on different tests. There are uncertainty and disagreements about the testing for viral RNA. Whom to test is an unanswered question. The common opinion is to test patients likely to have COVID-19; also the health-care workers and public health officials have the priority of testing.

National authorities have built guidelines for whom and when to test and they are updating them in the light of new information gathered over time.[9] They require testing everyone with symptoms so that can diagnose the infection early in time and control the spread successfully.

By evaluating predictors for SARS-CoV-2 infection, using exposure risk factors, demographic variables, clinical findings and clinical test results, it can be possible to identify subjects at high risk of COVID-19. Low leukocytes, low lymphocytes, higher body temperature, higher respiratory rate, gastrointestinal symptoms, and decreased sputum production were highly associated with a positive SARS-CoV-2 test. At this moment in time of a pandemic, these findings are very sensitive. But to confirm the infection whenever PCR is available, PCR should be performed as it is the gold standard method.[4]

Serological tests

According to European Union (EU) recommendations,[20] as an important part of the management of the pandemic and to slow it down, it is crucial to conduct accurate and well-timed COVID-19 laboratory testing. Health-care facilities take support from laboratory testing for infection control strategies and patient management. And with the help of these tests, the asymptomatic cases which could spread the virus unless isolated can be detected. ECDC and WHO recommend molecular tests which detect the SARS-CoV-2 virus RNA. This is the current test strategy for COVID-19 diagnosis. To perform these tests well-equipped laboratories, highly skilled technologists, and multiple reagents are required. For that reason, access to reliable rapid diagnostic tests such as rapid antigen tests or blood tests for Ab against the SARS-CoV-2 virus will sure be an essential step for monitoring the spread of the virus. That could reduce the pressure on laboratories and increase testing capacity to meet the most urgent medical and public health needs.

Direct SARS-CoV-2 antigen detecting and indirect antibody detecting tests are the two types of COVID-19 rapid tests currently in use. By using antigen detection tests, we detect the viral components in nasopharyngeal samples and by antibody tests detect the Ab in serum as the marker of the immune response against the virus. We have the experience of currently used antigen-based rapid diagnostic tests for other respiratory diseases. The sensitivity of these tests might be so variable, which means half or more of infected patients might be missed or false-positive results could occur by such tests, depending on the group of patients tested. On the other hand, the clinical value of the Ab is stick with the antibody responses of the host during the infection period. Since SARS-CoV-2 is a newly emerging virus, the antibody response in COVID-19 patients is still mostly unknown. Based on current data, rapid diagnostic tests detecting neither antigen nor antibody is the first choice of WHO for diagnosis but is recommended to be used for surveillance.[21]

Governments are looking to order millions of antibody tests that the medical diagnostic companies are still struggling to produce. COVID-19 Rapid Tests detect IgG and IgM Ab to SARS-CoV-2 in human whole blood, serum, and plasma samples qualitatively. These tests apply lateral flow immuno-chromatography and are used to assist in the diagnosis of SARS-CoV-2 infections. The IgM-IgG combined assays have better utility and sensitivity compared with single IgM or IgG tests.[22]

The rapid diagnostic tests may be less accurate and less sensitive than laboratory-performed diagnostic tests. Their performance results are not the same as in routine laboratory practice compared with the studies done by the manufacturer for the purpose of CE-marking. Therefore, clinical validation of these rapid tests for COVID-19 in real life should be done before routine as an independent diagnostic test. And that should be done by comparing it with the gold standard test in a sufficient number of target populations. Afterward can be used for the rapid screening of SARS-CoV-2 symptomatic or asymptomatic carriers in hospitals, clinics, and test laboratories.[22]

Detecting Ab against SARS-CoV-2 will be one of the most important goals. These serological analyses are critically important to determine seroprevalence so that we can identify highly reactive human donors. They will also support the screening of health care workers to identify the ones who are already immune. Comparing to PCR, the basic advantages of serological tests are their faster turn-around time, high output, and less workload.

For antibody testing of SARS-CoV-2, different enzyme-linked immunosorbent assay (ELISA) kits based on recombinant nucleocapsid protein and spike protein are used. The spike protein is the main antigen that brings out neutralizing Ab because this protein is the only protein on the viral surface that is responsible for entry into the host cell.[23]

The first larger study on the host humoral response against SARS-CoV-2 has shown that humoral response to SARS-CoV-2 can aid in the diagnosis of COVID-19, including subclinical cases.[24] In this study, IgA, IgM, and IgG response used an ELISA based assay on the recombinant viral nucleocapsid protein was analyzed in 208 plasma samples from 82 confirmed and 58 probable cases. The median duration of IgM and IgA antibody detection was 5 days (interquartile range [IQR] 3–6), while IgG was detected on 14 days (IQR 10–18) after

symptom onset, with a positive rate of 85.4%, 92.7%, and 77.9%, respectively. The detection efficiency by IgM ELISA was higher than that of qPCR after 5.5 days of onset of symptoms. Zhao *et al*. analyzed the dynamics of Ab with the disease progress on a group of 173 patients with SARS-CoV-2 infection. Their serial plasma samples (n = 535) collected during the hospitalization were tested for total Ab, IgM, and IgG against SARS-CoV-2. Among 173 patients, the seroconversion rate for Ab, IgM, and IgG was 93.1%, 82.7%, and 64.7%, respectively. The reason for the negative antibody findings in 12 patients might be due to the lack of blood samples at the later stage of illness. The median seroconversion time for Ab, IgM, and then IgG were day-11, day-12, and day-14, separately.[25]

Antibody testing is not useful in the emergence of acute illness as a consequence of that natural delay. We are not sure whether infected individuals who recover from SARS-CoV-2, fully or partially, will later be protected from SARS-CoV-2 infection or how long the protective immunity may last. There are a few important facts that antibody testing for SARS–CoV-2 may be useful. The first one is contact tracing, which can also be done by RNA-based tests; next is the serologic surveillance of the population, and last of all is the identification of the people who are already immune. If there is a protective immunity, serological information can be used to give return to work decisions, especially for health-care professionals. It may also be possible to use serologic testing to determine donors as a source for therapeutic plasma. Antibody testing can also be used in research studies to set the limits of the sensitivity of PCR analysis. And finally, serologic testing can be used diagnostically to test viral RNA-negative individuals presenting late in their illness.

Conclusion

Confirmation of the case, according to the national recommendations, provides supportive therapy to the patient and by detecting their contacts arrange infection prevention and control. Ideal guidance in current circumstances is what we should do with the available tests today. National health-care authorities should carefully consider what the most appropriate test is, and for whom and when.

In summary, under the present circumstances both molecular and serological tests should be beneficial. It is obvious that as the number of tests available increases other difficulties become evident. The performance of the tests varies according to the sample type, the original design of the test and the potential mutation of the virus. The sensitivity and the specificity of the tests can differ depending upon the sample type and date of sample collection. Optimization of a new test for a new virus needs many steps including collaborative clinical and laboratory trials; however, during the pandemic situation it is hard to establish such studies. More tests used in the field will provide more data gathered and a better understanding will be produced, but it needs time and practice. New point of care tests which are more rapid are under development and the widely usage of them will be crucial especially for the frontline health care providers. It should be taken into account that the tests must be appropriately validated before putting to use which will be the limitation of these tests.

The potential mutation of the virus is another challenge so that it will be necessary to sequence periodically in order to detect the possible changes in primer and probe binding regions. The good point is, the technologies we have are sufficient theoretically to do these studies but what we have is the serious time constraints and emergency conditions.

Last but not least as the number of tests performed and the turnaround time of the test decreases it will provide better management of both patients and health care workers. Except rapid tests which have the disadvantages mentioned lately, there are also limitations of testing capacity of the laboratories and the manufacturing capacity for diagnostic kits.

To provide powerful testing strategy rapid and accurate diagnostic tests are needed for which active collaboration between the laboratories and industry sectors is essential.

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Conflicts of interest
There are no conflicts of interest.

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