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Review / Derleme

Rational Use of COVID-19 Virological Tests in Intensive Care Unit

Yoğun Bakım Ünitesinde COVID-19 Virolojik Testlerinin Akılcı Kullanımı

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ABSTRACT

The ongoing COVID-19 pandemic reminded once again that microbiological diagnostic methods are irreplaceable in both diagnosing and detecting asymptomatic persons. At present, real-time reverse transcriptase polymerase chain reaction (RT-PCR) is the gold standard method for diagnosing COVID-19, but the test's accuracy varies in sample quality. Especially in the last stages of the disease, negative results of nasopharyngeal or oropharyngeal swab samples or rapid antigen tests do not necessarily mean that these patients do not carry the virus. Considering that a significant number of COVID-19 patients need intensive care and mechanical ventilation in the late period, which sample should be taken from where and when should be evaluated. Lower respiratory tract samples have a more significant chance of finding viral RNA than upper respiratory tract samples. Technical recommendations and the virological diagnostic methodologies and used in the intensive care unit of patients infected with SARS-CoV-2 are summarized in this article. We aimed to emphasize the need to get a sample from the right place at the right time for a reliable virological diagnosis.

Keywords: COVID-19, intensive care, microbiologic laboratory methods, SARS-CoV-2

ÖZ

Koronavirüs hastalığı-19 (COVID-19) pandemisi gerek tanıda gerekse asemptomatik kişilerin tespitinde mikrobiyolojik tanı yöntemlerinin vazgeçilmez olduğunu bir kez daha hatırlatmıştır. Günümüzde gerçek zamanlı revers transkriptaz polimeraz zincir reaksiyonu halen COVID-19 tanısı için altın standart tanı yöntemi olarak kabul edilmektedir, ancak testin doğruluğu alınan örneğin zamanlamasına ve kalitesine göre değişebilmektedir. Özellikle hastalığın ileri evrelerinde nazofarengeal veya orofarengeal sürüntü örneklerinin ya da hızlı antijen testlerinin negatif olarak sonuçlanması bu hastaların kesin olarak virüsü taşımadıkları anlamına gelmez. Ciddi sayıda COVID-19 hastasının geç dönemde yoğun bakım ve mekanik ventilasyona ihtiyacı olduğu düşünüldüğünde, nereden ve ne zaman numune alınması gerektiği iyi değerlendirilmelidir. Alt solunum yolu örneklerinde viral RNA bulma şansı üst solunum yolu örneklerine göre daha fazladır. SARS-CoV-2 ile enfekte hastaların yoğun bakım ünitesinde kullanılan virolojik tanı metodolojileri ve teknik öneriler bu yazıda özetlendi. Amacımız, güvenilir bir virolojik tanı için doğru zamanda doğru yerden örnek alınması gereksinimini vurgulamaktır.

Anahtar sözcükler: COVID-19, mikrobiyolojik laboratuvar tanı yöntemleri, SARS-CoV-2, yoğun bakım

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Introduction

The algorithm of microbiological test criteria and the performing of methods that will boost the reliability of the tests are two of the most effective alternatives for preventing the spread of SARS-CoV-2, finding and isolating contagious people, and treating those with symptoms.[1] The virus can be identified in patients' respiratory secretions 1-2 days before symptoms appear and 2 weeks after they appear.[2] SARS-CoV-2 screening tests are based on two key ideas. The virus isolation comes first, followed by determining the infection's host response.[3] There are several known virological tests for the isolation of the virus. Of these cell cultures and sequence analysis methods are specific procedures that are not suitable for routine use. Cell culture has high-quality virological tests but is not indicated as a standard diagnostic technique. It is suggested for researchers looking into the virus's properties, the modifications it has undergone, and the production of vaccines, disinfectants, and antiviral drugs.[4] Sequence analysis is recommended to be performed at frequent intervals to discover changes in the virus over time and mutations.[5] At present, the gold standard for routine practice for diagnosing and confirming Coronavirus 2019 disease (COVID-19) are nucleic acid amplification tests, particularly real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays.[6] These assays consist of the forward primer, reverse primer, and probe used together for amplification of the signals from the virus within a sample.[7] The test has a specificity of >95%, higher than its sensitivity (70%). Only 30-60% of clinically and radiologically suspected COVID-19 cases have a positive RT-PCR result. Most important for a definitive diagnosis of COVID-19 is identifying SARS-CoV-2 RNA in nasopharyngeal swab samples by RT-PCR[8] and as an alternative use of gargle and mouthwash samples.[9] The accuracy of the test depends on the sample quality, particularly the period of infection, the degree of virus proliferation, and the degree of virus clearance. RT-PCR assays can use serum, plasma (EDTA or citrate), whole blood, or saliva. The virus could also be detected in whole blood, serum, urine, and feces samples. But in upper respiratory tract samples, the maximum positivity occurs 3-21 days after initiation; this period is longer in lower respiratory tract samples. Therefore, deep tracheal aspirate or bronchoalveolar lavage samples should be preferred in mechanical ventilation.[10] The positive rate in stool samples is roughly 30%, although the rate in blood samples is substantially lower,[11] testing them is not recommended for routine use.

In addition, it seems helpful sending of RT-PCR assays from the patients at certain intervals, both in terms of disease progression and assessment of the possibility of transmission of the infections to personnel. Even more quantitative viral load monitoring in lower respiratory tract samples aids in illness progression evaluation, especially in low viral load cases.[12] But this advantage of RT-PCR assays for semi-quantitative measurement of viral load over the Cycle threshold (Ct) value is not well known by clinicians. This value is the number of cycles necessary for the fluorescent signal to cross the threshold. The quantity of target nucleic acid in a sample is inversely related to the Ct value, and higher Ct values indicate lower viral loads.[13] Many studies have reported Ct values for RT-PCR to determine the amount of SARS-CoV-2 RNA in respiratory specimens. Some have even found a correlation between SARS-CoV-2 Ct values and clinical outcomes.[14-16] Higher viral loads were found to be linked to more severe diseases.[14] On the other hand, some research showed no correlation between viral load and illness severity.[17] Although this topic is still debatable, it is undeniable that higher viral loads enhance the risk of transmission. More research is needed to identify the quantity of SARS-CoV-2 RNA in the lower respiratory sample of ICU patients. Furthermore, viral Ct values were discovered to be linked to computed tomography findings.[18]

The importance of a positive RT-PCR test is more significant.[19] A negative PCR test usually indicates that the person is not infected, but it does not rule out the possibility of infection (Table 1). Some studies report that PCR false-negative rates range from 2% to 29%, and negative RT-PCR tests were positive at repeat testing.[19] Contact history, clinical symptoms, radiological results, clinical examination, and biochemistry levels should be considered when evaluating suspicious cases. The factors contributing to a false-negative result should be clearly understood (Table 1) and should repeat the test within 24-48 h.[20] In cases when the second test is likewise negative, a non-COVID-19 diagnosis should be ruled out.[21] Antibody testing can help patients with a strong suspicion of COVID-19. The question of transmission risk and the safe period of self-isolation is raised by persistently or intermittently PCR-positive persons.[22] Nevertheless, it would be more accurate to do a study with this in mind on pure critical care patients and make comments based on it. Additional laboratory tests using standard RT-PCR methods are needed to assess the infection risk of transmission in people with persistent viral RNA shedding.[23]

It's just as vital to keep and transport the sample correctly to collect it accurately.[24] Clinical samples should transport as quickly as feasible to the laboratory, can be held at 2-8°C for up to 72 h maximum; if a delay of more than 5 days is foreseen in sending samples, they should freeze them, preferably at -70°C.[25] Due to the potential to generate aerosols or droplets of laboratory procedures, the Centers for Disease Control and Prevention recommends utilizing Class II biological safety cabinets. They provide three levels of protection: people protection, sample protection, and

Table 1. Quantitative RT-PCR results evaluation

False-negative RT-PCR causes	False-positive RT-PCR causes
• Inappropriate clinical sample quality	• None
• Inappropriate timing of sampling	
• Improper transport and storage conditions of the clinical specimen	
• Pipetting and processing mistakes	
• Giving antiviral before testing	

RT-PCR: Reverse transcriptase polymerase chain reaction.

environmental protection. Higher Biosafety Level should handle SARS-CoV-2 cell cultures or animal models.[26]

In terms of monitoring, serological assays such as antigen and antibody testing are crucial for tracking and managing the virus's course, particularly in detecting past infections.[27] These assays look for viral proteins that are particular to that virus. Antigen detection tests (Ag-RDT) are highly beneficial in clinics since they may deliver a diagnosis in as little as 15-30 min at the patient's bedside and do not necessitate the employment of qualified specialists. The diagnostic value of Ag-RDT is lower than the RT-PCR test, but negative antigen detection tests (Ag-RDT) could reliably rule out infection.[28] False-negative results may be seen when the viral load is lower.[10] It may yield more reliable results if done 1-2 days before the onset of symptoms and within 5-6 days following the signs. If the patient's antigen tests positive for SARS-CoV-2, it must be confirmed by PCR (Fig. 1). The patient should consider positive and isolated until the RT-PCR test is completed.[29] False positivity is possible due to cross-reactivity with other Coronaviruses; thus, results must be double-checked.[30] Independent evaluation of different rapid Ag-RDTs has shown their sensitivity ranges between 70% and 90% (lower confidence limits 50-80%) in symptomatic individuals.[31] Still, it deteriorates remarkably (<50%) in asymptomatic close contacts[32] in those with low nasopharyngeal viral loads.[33]

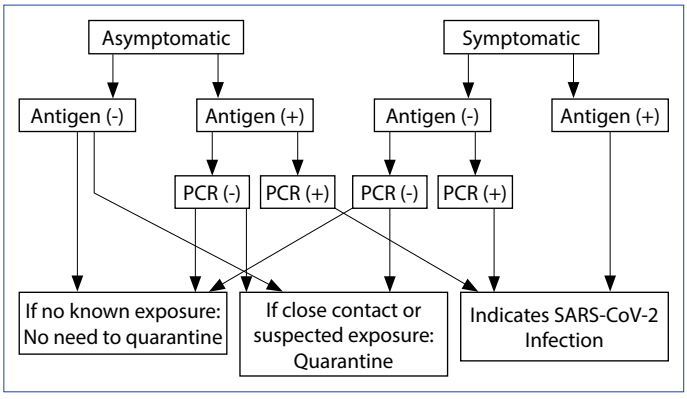

Figure 1. Antigen test algorithm; adopted from "Interim guidance for antigen testing for SARS-CoV-2, updated September 9, 2021".

Table 2. Use of serological tests for COVID-19 infection

- Determining the seroprevalence of the infection in the population
- Screening of asymptomatic people
- Monitoring of contact people
- Confirmation of the diagnosis in viral RNA-negative patients late in the disease
- Identifying individuals who may be a source of neutralizing antibodies
- Utilization of serological test results in susceptibility studies of PCR tests
- Calculation of statistics on the true extent of the pandemic
- Determining disease control policies, the spread of infection, and the relaxation of existing restrictions on social distancing, the decision to return to work for those at risk of re-exposure to SARS-CoV-2.

Several commercially prepared serological assays were developed.[34] While some tests only detect total immunoglobulin, others can detect both IgM and IgG in two different assays. Automated chemiluminescent immunoassay (IA), which can detect IgM and IgG for SARS-CoV-2 infection, ELISA-based technologies, and Lateral Flow IA with rapid findings are the most common IAs. These technologies target recombinant nucleocapsid protein and spike protein. They cannot substitute RT-PCR in determining the presence or absence of acute SARS-CoV-2 infection,[34,35] but their positivity indicates that the individual has previously been infected with SARS-CoV-2[35] (Table 2). They can especially help diagnose individuals who have had negative RT-PCR tests despite having a high level of clinical suspicion. Data on the kinetics of viral neutralizing antibody (NAb) responses are essential since virus-NAb is anticipated to be a critical correlate of COVID-19 protection.[36] The result depends on the timing of infection; and moreover, still unknown the mechanisms of protective immunity, as well as the length of time that such immunity lasts. Antibody titers often peak 30-40 days following the onset of sickness, followed by a decline in antibody titers. Even after NAb levels have declined below the detectable threshold, immunological memory can result in antibody responses when re-exposure to the virus. Although antibodies do not give sterilizing immunity, they can guard against reinfection, leading to severe illness.[37,38]

Conclusion

SARS-CoV-2 laboratory diagnosis is critical for disease management, but the accuracy of test results can vary by the time of symptoms; thus, a thorough patient anamnesis is essential. Periodic RT-PCR controls seem to be beneficial in terms of both disease progression and personal safety. COVID-19 still requires the development of more precise microbiological procedures. Superfluous testing raises costs, and the medicinal value to the patient appears to be disputed.

Disclosures

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