American Journal of Physical Medicine & Rehabilitation Articles Ahead of Print
DOI: 10.1097/PHM.0000000000001471

Interpreting the COVID-19 Test Results: a Guide for Physiatrists

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Conflicts of interests: No disclosure.

Acknowledgement: None

Dear editor,

On March 11, 2020, the World Health Organization declared the outbreak a pandemic.[1, 2] Although various novel coronavirus disease 2019 (COVID-19) diagnostic methods are being used worldwide, the advantages and disadvantages of each testing method and the cautions needed when interpreting the results of each method are not well known to the physiatrists. For proper management and containment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)transmission in the rehabilitation department of the hospital, physiatrists need to understand the advantages and disadvantages of the various diagnostic tests available and the cautions required in interpreting the results. Therefore, in this review, we discuss the various diagnostic methods for COVID-19 and the caution needed when interpreting the test results.

Classification of the Diagnostic Methods for novel coronavirus disease 2019 (COVID-19)

Three types of diagnostic methods are currently available for COVID-19, and these include a molecular diagnostic method (real-time polymerase chain reaction, RT-PCR), a culture method, and an antigen-antibody test method (Table 1). The RT-PCR-based tests for COVID-19 are of two types: pancoronavirus RT-PCR and real-time reverse transcription polymerase chain reaction (rRT-PCR).

Pancoronavirus RT-PCR Assay

The pancoronavirus RT-PCR assay first analyzes the suspected clinical sample for all the coronaviruses. If a positive reaction is detected in the test, a second test is performed using gene sequencing to determine whether the coronavirus is SARS-CoV-2. Therefore, this assay can take

up to 24 h to confirm COVID-19. Despite the accuracy of the pancoronavirus RT-PCR test, this assay presents several major limitations under the current pandemic situation due to the time and effort required for diagnosis. However, the pancoronavirus RT-PCR test could be used to rule out the possibility of false negative results in the real-time reverse transcription polymerase chain reaction (rRT-PCR) method.

rRT-PCR Assay

Currently, *rRT-PCR* is the most widely used diagnostic method for COVID-19. To understand the principle of the assay and the choice of primer sets used, some basic knowledge of COVID-19 biology is necessary. The SARS-CoV-2 genome encodes four structural proteins. The spike surface glycoprotein (S) mediates specific binding to the host cell receptors, the nucleocapsid (N) protein binds to the coronavirus RNA genome to make the nucleocapsid, the membrane (M) protein is the main structural protein that connects between the membrane and the capsid, and the small envelope (E) protein which is involved in the assembly and budding process of the coronavirus.[3] Among them, the genes for the N and E proteins are used as the targets for amplification in the rRT-PCR assay combined with the open reading frame 1 (ORF1) ab, and the RNA-dependent RNA polymerase (RdRP) gene.

Most countries currently use rRT-PCR-based assays for the detection of COVID-19 infection. Examples of a few countries and the target genes assayed are as follows: China (ORF1 ab, N), Germany (RdRP, E, N), Hong Kong (OLRF1b-nsp14, N), Japan (Pancoronavirus and multiple targets, S), Thailand (N), the United States (three targets in N), and France (two targets in RdRP). These countries have published their molecular diagnostic protocols and the primer/probe sequences on the World Health Organization website.[4] Examples of RT-PCR

diagnostic kits based on the aforementioned genes that are currently used in South Korea and the United States are listed in Supplementary 1 (Supplemental Digital Content 1, http://links.lww.com/PHM/B10). Since rRT-PCR-based assays usually detect only 2–3 of these genes, the assay allows for rapid testing and diagnosis. However, interpreting the results may be challenging and requires attention.

Notes on Interpreting rRT-PCR Results

Firstly, because rRT-PCR methods usually detect only 2–3 of these genes, it has the advantage of rapid diagnosis. However, given that mutations occur frequently in SARS-CoV-2, the possibility of false negatives in the diagnosis of COVID-19 may be a disadvantage of rRT-PCR -based methods. To overcome this drawback, it may be helpful to simultaneously use two or more rRT-PCR diagnostic kits that detect different viral genes.

Secondly, the diagnosis of COVID-19 using rRT-PCR methods is not clearly classified as positive or negative, instead the diagnosis is made based on the threshold cycle (Ct) value. Ct is defined as the cycle number when the sample fluorescence exceeds a chosen threshold above the calculated background fluorescence.[5] In other words, the lower the Ct value of a specific gene, the more the gene exists in the sample. However, the problem with a Ct-based diagnosis is that there is no absolute or constant Ct cut-off value, and Ct cut-off values are different for each diagnostic reagent even for the same gene. For example, although there are differences according to diagnostic reagents, a sample is usually judged positive for COVID-19 based on a Ct value of 35. Although the Ct value in a rRT-PCR test is relatively accurate, error of 1~2 cycles are not uncommon in a Ct value depending on various factors, including the skill of the examiner. Therefore, when there is ambiguity in the Ct value, such as 34~36, the result may be interpreted

as false negative or false positive depending on the Ct cut-off value. Furthermore, because the Ct value is inversely proportional to the amount of the target gene, there is also the disadvantage of a sample being interpreted as false negative in the early stages of COVID-19 infection without large amounts of virus multiplication, or depending on the accuracy of the swab. Therefore, to overcome these limitations of the rRT-PCR method, the following strategies can be adopted.

One way to judge ambiguous rRT-PCR results may be through detailed standard operating procedures performed by a centralized decision-making body consisting of specialists authorized by the government. In addition, since the Ct value is a non-standardized value and depends on the diagnostic reagent used, it may be necessary to standardize the Ct value according to the product for the same virus concentration. These standards could be optimized and set by government organizations, such as the Center for Disease Control and Prevention (CDC). Moreover, it is important to obtain two or more swabs from two or more sites (nasopharyngeal and throat swab) from each patient and perform consecutive tests (while the suspected patient is kept in isolation) to resolve a false negative result which may have been caused by early stage of COVID-19 infection or inaccuracy of the swabbing method.

A recent study from China reported over 50% false negative cases using rRT-PCR tests for COVID-19. However, considering the accuracy of rRT-PCR, these high false negative results may be due to problems with the Ct cut-off value, gene selection, accuracy of swab, or use of reagents that were produced at an early stage of the COVID-19 spread and had not been verified for accuracy. The Korean society for laboratory medicine reported that, although different for each reagent, rRT-PCR methods have a diagnostic accuracy of approximately 95% for COVID-19.[3] The rRT-PCR-based SARS-CoV-2 kit (Cobas®, Roche) which has been approved by the United States Food and Drug Administration (FDA), has also been reported to have ≥ 95%

diagnostic accuracy. However, despite the accuracy of rRT-PCR tests, it is important that clinicians always interpret false negative rRT-PCR test results with caution because false negative results can be caused by various factors as mentioned earlier.

Viral Isolation Using Viral Culture Method

Although it is possible to detect new pathogens through genetic analysis, it is necessary to establish causation of the disease according to the Koch's Postulates. SARS-CoV-2 was first isolated through cell culture (Vero E6 and Huh7 cells) using bronchoalveolar lavage fluid from COVID-19 patients in intensive care units in China.[4] The identity of SARS-CoV-2 was then verified using immunofluorescence microscopy with cross-reactive viral N antibody, electron microscopy, and genetic analysis.[4]

There are two main methods of viral isolation following viral culture. In the first method, viral isolation is performed using the traditional cell culture method, which is still accepted as the gold standard method. Although this test can confirm the presence of virus through the observation of cytopathic effects, as in the case of SARS-CoV-2 isolation and verification process mentioned earlier, additional methods such as immunofluorescent staining must be performed. These methods can take between 2–14 days to identify the virus.

To overcome the limitation of the traditional cell culture method, a rapid shell vial cell culture method, which improves virus cell infection through a centrifugation step, was developed.[6] Although the time required for viral isolation using this method is shorter than with the traditional cell culture method, this method still takes 24–72 h, thereby limiting its diagnostic application in the field where rapid identification is needed. In addition, both the methods present

considerable risk of infection for the examiner and should only be performed when special facilities are available. Nevertheless, viral isolation through cell culture is essential for molecular biological research on new infectious diseases, and for the development and evaluation of therapeutic agents such as antibodies, vaccines, and diagnostic agents. Additionally, viral isolation using viral culture method can be used to confirm a diagnosis and to exclude a false negative result obtained through other methods such as rRT-PCR.

Antigen-Antibody Test

The antigen-antibody tests are based on immunochromatography. Recently, researchers from China reported the development of a diagnostic method to detect immunoglobulin M (IgM) and immunoglobulin G (IgG) against SARS-CoV-2, with sensitivity and specificity of 88.66% and 90.63%, respectively.[7] Outside of this report, the accuracy of an antigen-antibody test is generally reported to be 50–70%.[8] While the antigen-antibody test has some disadvantages, it has the following advantages. The antigen-antibody test methods are generally fast and simple. The results are available in about 10 min. In addition, this method can be performed quickly and easily even with a single drop of blood. As a result, this method is particularly useful for clinicians in the field. However, the following points need to be noted when interpreting the results of the antigen-antibody test.

Firstly, COVID-19 may be difficult to diagnose at an early stage of infection because a certain time period (5-14 days) is needed by the host to produce IgM and IgG antibodies against the virus. Therefore, clinicians should exercise caution when interpreting false negative results during early stages of COVID-19 infection. Secondly, since cross-validation with other viral infections, including influenza, has not been completely performed, it may be necessary to use

this in combination with other molecular diagnostic tests.[7] Therefore, clinicians are recommended not to rely solely on the antigen-antibody test for diagnosing or excluding COVID-19 infection. It is suggested that the antigen-antibody method be used as a complementary diagnostic tool for rRT-PCR. Considering the characteristics of the antigen-antibody test, it may be useful in the following scenarios: to confirm COVID-19 infection when false negative results are suspected in rRT-PCR, to investigate how much COVID-19 infection has spread in the community, or to determine whether immunity has been acquired in the community.

Here, we reviewed representative COVID-19 diagnostic methods. Although various methods are used in different countries of diagnosing COVID-19, the advantages and disadvantages of the each test method and cautions to be exercised when interpreting the results are not well known to the physiatrists. We hope that this review of the various COVID-19 test methods will help clinicians in the field make the right decisions regarding the choice of test and interpretation of the results.

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Table 1. Testing methods for coronavirus disease 2019 (COVID-19)

	RT-PCR	Antigen-antibody test	Viral culture
Details	Real-time reverse transcription polymerase chain reaction (RT-PCR), gene detection	Serum testing, immunological testing	Viral isolation using cell culture
Specimen collection	Nasopharyngeal and throat swab, bronchoalveolar lavage fluid	Blood, serum	Nasopharyngeal and throat swab, bronchoalveolar lavage fluid
Main target of testing	Genes	Antibodies	Virus
Advantages	High accuracy (97%)	Rapid testing (results within around 10 min)	Gold standard
Disadvantages	Requires strictly controlled facilities, such as a Biosafety level 3 (BL3) or higher laboratory	Low accuracy (50–70%; some manufacturers claim 90%)	Requires strictly controlled facilities, infection risk of examiner, needs extra-process, such as immunofluorescence staining
Time to analyze results	6 h	10 min	3~10 days
Purpose	Confirmation of COVID-19 diagnosis	Checking community infection and antibody formation	Confirmation of COVID-19 diagnosis
Characteristics	This method is currently used to confirm COVID-19 diagnosis in most countries. The US FDA has approved an automated testing device, Cobas® SARS-CoV-2 kit, manufactured by Roche.	Although widely developed in the form of a rapid diagnostic kit, this method is not yet being used to confirm COVID-19 diagnosis.	