RT-PCR in Diagnosis of COVID-19

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Corona virus disease 19 (COVID-19) demonstrates greater impact on global public health, economy, education, tourism and sports etc. So, rapid and accurate investigation of COVID-19 is very important for proper management. We can diagnose COVID-19 by detecting the presence of Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) in the samples. SARS-CoV-2 can be identifying either by the presence of the virus or antibodies produced in response to its infection.[1] COVID-19 testing include the test for the current infection or past infection. These tests confirm the presence of a virus in the body (current infection) or any past infection that causes COVID-19. An antibody test will show whether or not someone has developed antibodies against COVID-19 after exposure or vaccination. This test cannot confirm the active infection. Early and accurate detection of COVID-19 will help to trace and control the source of infections.[2]

There are two main types of diagnostic tests for COVID-19 infection. These are antigen tests and molecular tests. Antigen tests are rapid tests, which detect certain proteins on the surface or in the virus, are economical, efficient, and fast. Many of these tests have been authorized by FDA for emergency use, some even in the habitat environment. They can be used to make a clinical diagnosis in symptomatic patients in the first five days of symptoms. Molecular tests are more accurate and current standard for diagnosing COVID-19. These tests look for the genetic materials of virus. There are multiple types of molecular tests. Among them polymerase chain reaction (PCR) test is most commonly used and accurate.[3] PCR is an artificial method of cloning or amplification of selected fragment of DNA. It is very fast and even a trace amount of target DNA can be amplified. The average PCR involves 30-35 cycles and it can produce millions to billions copies of target DNA within few hours. Reverse transcription (RT) is a process of synthesis of DNA from RNA catalyzed by the enzyme reverse transcriptase.[4]

Real-time reverse transcription polymerase chain reaction (rRT-PCR) test is used for detection of nucleic acid from SARS-CoV-2. RT-PCR first uses reverse transcription to obtain DNA from viral RNA, followed by PCR to amplify that DNA. Real time detection of genetic materials makes it more sensitive test; as a result, rRT-PCR is the most commonly used diagnostic test for detection of COVID-19 within few hours to days.[5]

Sensitivity may be defined as the capacity of a test to identify all infected people and specificity is the ability of a test to detect a particular antigen.[2] Sensitivity of rapid molecular tests varied according to test brand. Sensitivity of RT-PCR test is determined by using data from different studies.[6] In one study, the average sensitivity of ID NOW was 73.0% (95% CI- 66.8% to 78.4%) and average specificity 99.7% (95% CI- 98.7% to 99.9%; 4 evaluations; 812 samples, 222 cases). In case of Xpert Xpress, the average sensitivity was 100% (95% CI- 88.1% to 100%) and average specificity 97.2% (95% CI- 89.4% to

99.3%; 2 evaluations; 100 samples, 29 cases).[6] Dinnes et al.[6] showed higher sensitivity in the first week after symptom onset (78.3%, 95% CI- 71.1% to 84.1%; 26 evaluations; 5769 samples, 2320 cases) than in the second week of symptoms (51.0%, 95% CI- 40.8% to 61.0%; 22 evaluations; 935 samples, 692 cases). Sensitivity was high in those with cycle threshold (Ct) values on PCR ≤25 (94.5%, 95% CI 91.0% to 96.7%; 36 evaluations; 2613 cases) compared to those with Ct values >25 (40.7%, 95% CI 31.8% to 50.3%; 36 evaluations; 2632 cases). A cycle threshold of 20 cycles would be adequate to detect SARS-CoV-2 in a highly infective person. Cycle thresholds above 34 cycle thresholds increase the chance of giving false positive results.[7] Xu et al.[8] mentioned highest sensitivity (100%) of RT-PCR test at week one, followed by 89.3%, 66.1%, 32.1%, 5.4% and 00% by six week.

Regarding sample collection for RT-PCR test, Centers for The Disease Control and Prevention (CDC) recommends upper respiratory specimen. Samples may be collected from nasopharyngeal swab, throat swabs, deep airway material collected via suction catheter, sputum or saliva. As nasal and throat swabs saliva may be an effective sample that may reduce the infection risk for collectors due to less chance of contamination. Saliva can be collected by the quarantined people themselves and that is more comfortable for other patients also. In another study[8] explained the sensitivity and specificity of the saliva sample were 84.2% (95% CI- 60.4% to 96.6%), and 98.9% (95% CI- 96.1% to 99.9%), respectively. For COVID-19 diagnosis the nasopharyngeal swab remains the most widely used sample. Its two main drawbacks are its technical difficulty and painfulness. Well-trained testing teams should help increase the sensitivity of the test and make it less unpleasant. From a diagnostic point of view, it is important to note that nasal and throat swabs seem less suitable for diagnosis, since these materials contain considerably less viral RNA and the virus may escape detection if only these materials are tested. The overall sensitivity were 100.0%, 67.5% and 37.5% for nasopharyngeal swab, nasal swab and saliva swab samples.[9] The likelihood of detecting the virus depends on collection method and how much time has passed since infection. Tests performed with throat swabs are reliable only in the first week. Thereafter, the virus may abandon the throat and multiply in the lungs. In the second week, sputum or deep airways collection is preferred.[9] It was specified that, sensitivity of clinical samples by RT-PCR was 63% for nasal swab, 32% for pharyngeal swab, 72-75% for sputum, and 93-95% for Broncho-alveolar lavage.[10]

Some evidence showed that, a good proportion of new mild cases that were positive, after quarantine or discharge from hospital re-testing via RT-PCR are not infectious. They simply demolish harmless virus particles by their immune system. So, an international effort to standardize and periodically calibrate RT-PCR testing is hardly needed.[11]

In conclusion, the results of RT-PCR tests must be cautiously interpreted. Multiple samples collected from different sites of respiratory tract in different times are distinguished carefully. Sample from the lower respiratory tract should be tested in case of negative result in RT-PCR with clinical features suspicious for COVID-19. Proper sampling, good laboratory practice, using high-quality extraction and standard RT-PCR kit could improve the accuracy of results.

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