https://doi.org/10.36570/jduhs.2020.2.998

JDUHS

EDITORIAL

Laboratory Diagnosis of Novel Coronavirus Disease 2019 (COVID-19) Infection

Dr. Saqib H. Ansari

Pediatric Bone Marrow Transplant Physician, Children's Hospital Karachi

Correspondence to: Dr. Saqib H. Ansari, Email: muddasirsaqib@yahoo.com, ORCiD: 0000-0002-4209-6560

A cluster of pneumonia cases were reported to World Health Organization (WHO) on 31st December, 2019 from the city of Wuhan, China which were later identified to be caused by a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The outbreak spread globally and on 11th March 2020, WHO declared COVID-19 as a pandemic. There have been continuous efforts to optimize methods to screen and diagnose large proportion of coronavirus affected population. COVID-19 diagnostic approaches include real-time reverse transcription-PCR (RT-PCR) and serological diagnosis.

RT- PCR is the molecular test of choice: SARS-CoV-2 is an enveloped, positive-sense and a single-stranded RNA virus[2] and the molecular test of choice for its diagnosis remains to be RT-PCR. Viral cultures are not recommended as they take around 3 days to demonstrate cytopathic effects and also require biosafety level 3 facilities which are unavailable in most of the institutes.[1]

Specimen collection from the respiratory tract requires a single nasopharyngeal (NP) swab as it is well-tolerated by the patient and a safer option for the healthcare workers. The nasal swab is inserted deep into the nasal cavity which will potentially cause the patient to flinch, indicating that the swab has reached the correct site. Swab should be left inside for 10 seconds and then taken out after twirling it thrice. Due to coronavirus's ability of airborne transmission, use of personal protective equipment (PPE) is required. Viral (universal) transport medium should be used for transportation of the sample to the laboratory, preferably under refrigerated conditions. NP sample collection could miss early infections or at the later stages, the infection could be shifted to the lower tract which would require re-sampling such as sputum sampling or broncho-alveolar lavage.[3] Laboratory processing of the samples should be performed in a class II biological safety cabinet.

Envelope glycoprotein spike, nucleocapsid, helicase, envelope, open reading frame 1a (ORF1a) and ORF1b are few possible targets that could be used for PCR assays.[4] Various manufacturers utilize a combination of two regions to diagnose SARS-CoV-2. The test is confirmed positive when both of the regions are positive [4]. The indications for real-time RT-PCR testing for COVID 19 include confirmatory testing for suspected cases, screening close contact asymptomatic patients and differential diagnosis for individuals with undiagnosed respiratory syndromes.[5] RT-PCR is being widely used and has high sensitivity and specificity for SARS-CoV-2. Nonetheless, it is an expensive test which requires qualified professionals and infrastructure.[2] A critical issue related to RT-PCR is the possibility of false-negatives, many cases with high clinical suspect and characteristic computed tomography (CT) scan findings were not confirmed by PCR.[6] False-negative results could be due to inaccurate sampling procedures, early or late sample collection, inadequate handling and transportation of the sample, possible genetic mutation of the viral pathogen or due to intake of anti-viral medications before testing.[5] Efforts to ensure adequate sampling and handling, upholding laboratory processing standards and use of high-quality extraction and real-time RT-PCR kit would reduce the possibilities of inaccurate results.[6]

Serological diagnosis of COVID-19 includes the detection of IgM and IgG. Enzyme-Linked Immune Assay (ELISA) is used to detect these antibodies against nucleoprotein and spike proteins of SARS-CoV-2. ELISA is not an expensive test and has almost the same turnaround time as RT-PCR.[2] Many Rapid detection tests for IgM and IgG have also been manufactured with about 10-15 minutes of result timings; however, these tests have low specificity and sensitivity.[2]

Seroconversion in COVID-19 requires a period of 7-14 days. Serology could be utilized for epidemiological purposes or to determine immune status of asymptomatic individuals; however, they could produce inaccurate results in early period of infection.[3]

After establishing the diagnosis of COVID-19, additional laboratory testing could be utilized to assess the severity and monitor the progress and resolution of the disease. IL-6 and d-Dimer levels could be used to assess the severity of the illness. Glucose, CRP, thrombin time and fibrinogen levels have also been reported to be

higher in severe cases of COVID-19.[7]

COVID-19 diagnostic facilities and resources in Pakistan: During initial phases, the diagnostic services were being provided by very limited government and private institutions in bigger cities who had pre-established infrastructure and molecular diagnostic facilities. However, growing need for testing services all across the country for COVID-19 led to development and increased accessibility of services on emergent basis. Efforts were directed to procure equipment, advance infrastructure and assemble human resources to meet the needs and demands. Research institutes also aided by offering their educational premises for diagnostic purposes. As per the report released by National Institute of Health (NIH), Islamabad, Pakistan has 104 functional labs, majority of which, 30 are in Punjab, Sindh has 19 functional labs, Khyber Pakhtunkhwa has 14 functional labs, Islamabad has 12 functional labs, Baluchistan has 5 functional labs, Azad Jammu Kashmir has 3 functional labs, Gilgit Baltistan has also 3 functional labs, Armed forces has 14 functional labs while Strategic Plans Division Force has 4 functional labs.[8] Moreover, Pakistan has daily testing capacity of over 16,000 tests a day. Out of which, Sindh has 11,480 per day testing capacity, Punjab has 5810 per day testing capacity, Islamabad has 2756 per day testing capacity, Khyber Pakhtunkhwa has 1788 tests per day testing capacity, Baluchistan has 894 tests per day testing capacity, Gilgit Baltistan and Azad Jammu and Kashmir has 188 and 160 testing capacity per day respectively.[9]

Cost was another crucial hindrance in COVID-19 diagnosis, due to lack of laboratory regulatory authority in Pakistan. As the time passed and because of various government, private and international organizations' involvement, the costing has been contained to some extent. One of the recommendations to ensure accuracy for the diagnostic laboratories offering COVID-19 testing in Pakistan is to conduct internal audits by running controls and randomly sending out samples to other accredited laboratories for cross-checking. Another recommendation to improve overall diagnostic conditions in Pakistan is establishment of government authoritative bodies to comprehensively regulate diagnostic services and laboratories.

In conclusion, pandemic of COVID-19 has highlighted the significant role of laboratory diagnosis in management of diseases. RT-PCR is currently the gold-standard for the symptomatic and asymptomatic cases of COVID-19 which requires trained professionals to efficiently follow the guidelines and standards and ensure accurate test results.

REFERENCES

1. Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, et al. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. J Clin Microbiol 2020; 58:e00310-20. doi:10.1128/JCM.00310-20
2. Russo A, Minichini C, Starace M, Astorri R, Calo F, Coppola N. Current Status of Laboratory Diagnosis for COVID-19: A Narrative Review. Infect Drug Resist 2020;13:2657-2665. doi:10.2147/IDR.S264020
3. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. J Clin Microbiol 2020; 58 :e00512-20. doi:10.1128/JCM.00512-20
4. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal 2020; 10 :102-108. doi:10.1016/j.jpha.2020.03.001
5. Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, et al. Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. Ann Lab Med 2020 ;40 :351-60. doi:10.3343/alm.2020.40.5.351
6. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert Rev Mol Diagn* 2020;20:453. doi:10.1080/14737159.2020.175743
7. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol 2020;92 :791-6. doi:10.1002/jmv.25770
8. National Institute of Health (NIH). COVID – 19 Laboratory Capacity in Pakistan. Available at: http://covid.gov.pk/facilities/10%20June%202020%20Current%20Laboratory%20Testing%20Capacity%20for%20COVID.pdf Accessed: 25th August 2020
9. The Nation. Pakistan's testing capacity for COVID-19 is over 16,000 per day: WHO. Available at: https://nation.com.pk/09-May-2020/pakistan-s-testing-capacity-for-covid-19-is-over-16-000-per-day-who Accessed: 25th August 2020