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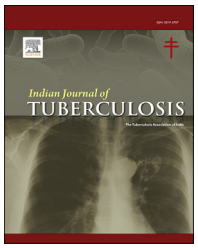

Original Article

Laboratory diagnosis of novel corona virus (2019-nCoV)-present and the future

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ABSTRACT

Background: In December 2019 a novel coronavirus SARS-CoV-2 emerged in the Hunan seafood market in Wuhan, China, and soon became a global health problem. Since its outbreak, SARS-CoV-2 has had a major impact on clinical diagnostic laboratories. The scientific community has quickly risen to the occasion and reports of new developments have arrived at an unprecedented scale. At present, there is a growing list of over 400 SARC-CoV-2 diagnostic tests either in development or approved for clinical use. This presentation reviews the current laboratory methods available for testing COVID- 19 in microbiology laboratories and also provides an insight into the future diagnostics approaches.

Methods: Proper respiratory specimen collected at the appropriate time and from the right anatomical site is critical in the accurate and timely diagnosis of SARSCoV2. While oropharyngeal and nasopharyngeal swabs are recommended for the detection of early infection, other lower respiratory tract specimens like the sputum and bronchoalveolar lavage are used for late detection and monitoring of patients with severe COVID-19 pneumonia.

Results and Conclusion: Real-time RT-PCR based molecular assay remains the test of choice for the etiological diagnosis of SARS-CoV-2 while serological tests are being introduced as supplementary tools. Finally, there is an urgent need for scaling up the diagnostic capacity by the introduction of reliable and accurate point-of-care tests which will assist in effective control of this outbreak. These assays can be used in the local hospitals and clinics bearing the burden of identifying and treating patients.

1. Introduction

Coronaviruses belong to family Coronaviridae which include four genera designated by Greek letters alpha, beta, delta, and gamma.[1] Of these only alphacoronaviruses and betacoronaviruses are known to cause disease in humans. Coronaviruses are so named because they look like halos (known as coronas) when viewed under the electron microscope. These spherical shaped enveloped viruses contain single-stranded 32 kilobases long positive-sense RNA. Coronaviruses have the largest known viral genome and can

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contain up to 10 open reading frames (ORFs). These viruses spread through the air and are responsible for about 10–30% of the colds worldwide. Since the isolation of the first coronavirus in 1937, Human coronaviruses (HCoVs) have been known to cause mild upper respiratory disease in humans until two highly pathogenic HCoVs-severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged from animal reservoirs in the 21st century. SARS-CoV and MERS-CoV caused a global epidemic with alarming morbidity and mortality.[2,3] In December 2019, a novel coronavirus named as 2019-novel coronavirus (2019-nCoV or SARS-CoV-2) was identified to cause atypical pneumonia in a cluster of patients linked to the wholesale market in Wuhan, China.[4] Soon 2019-nCoV was reported from almost all the countries in the world, suggesting the possibility of human-to-human transmissions and/or multiple spill-over events in different settings. On March 12th, 2020, WHO declared the 2019- nCoV outbreak as pandemic.[5] The spectrum of this disease in humans is yet to be fully determined. The incubation period of SARS-CoV-2 infection is almost similar to that of SARS. The reported mean incubation period of the disease is 5.2 days.[6] The reported symptoms include fever and cough reported early in the course of illness.[7,8] Infections are also characterized by dyspnea, respiratory distress, and a positive chest X-ray.[9] Lower respiratory symptoms often develop about 1 week from the onset of initial symptoms.[7] As of August 16, 2020, a total 21,294,845 confirmed cases and 761,779 deaths due to 2019-nCoV have been reported globally.[10] There is thus an urgent need for reliable diagnostic tests specific for confirming suspected cases, screening patients, and conducting virus surveillance.

2. Laboratory diagnosis

2.1. *Specimen collection*

Upper respiratory tract specimens collected within 5–6 days of the onset of the symptoms have demonstrated high viral loads. Thus, a nasopharyngeal and/or an oropharyngeal swab are recommended for initial screening and diagnosis of early infection.[11,12] Swabs should have flocked non-toxic synthetic fibers, such as polyester, as well as synthetic nylon handles.[13] Because of its safety and better tolerance by the patients, a single nasopharyngeal swab has become the specimen of choice. Wang et, al., in their recent study have reported that oropharyngeal swabs (n = 398) were used much more frequently than nasal swabs (n = 8) in China during the COVID-19 outbreak; however, they were able to detect SARS-CoV-2 RNA in only 32% of oropharyngeal swabs, which was significantly lower than the level in nasal swabs (63%).[14] In order to increase the sensitivity, the WHO recommends the collection of a combined nasopharyngeal and oropharyngeal swab.[15] However, an important aspect to consider while collecting both nasal and oral swabs either as independent specimens or together within a single aliquot of viral transport medium is that it may place potential stress on national and institutional supply chains. Once collected, swabs should be placed in vials containing Viral Transport Medium (VTM) and transported to the laboratory within 24–72 hours, ideally under refrigerated conditions (4–8 °C). If delays in transport are expected, the samples should be stored at −70 °C.

Lower respiratory tract specimens such as sputum and bronchoalveolar lavage are used for late detection and monitoring of patients with severe COVID-19 pneumonia.[6,16] A recent study concluded that bronchoalveolar lavages yield the highest SARS-CoV-2 RNA.[14] It should however be noted that this study did not compare/evaluate the use of nasopharyngeal or oropharyngeal swabs. In patients requiring emergent intubation, a lower respiratory tract specimen should be collected during the intubation procedure. Some patients with COVID-19 pneumonia have also demonstrated high viral loads in fecal material late in their clinical course.[17] The enteric involvement in patients with severe coronavirus infections has previously been established where SARS coronaviruses were isolated from the stool and were also demonstrated in enterocytes under the electron microscope.[18,19] Thus, in addition to direct respiratory samples, in advanced COVID-19 cases, rectal swabs may be preferred sample for diagnosis.[18]

2.2. *Assay selection*

Since its emergence the development of SARC-CoV-2 diagnostics has advanced rapidly. While most countries are relying on nucleic acid testing, there is an ongoing effort to develop serological tests.[20,21]

2.3. *Serology*

Immunoassays have been developed for the rapid detection of SARS-CoV-2 antigens or antibodies. These rapid point-of-care immunoassays are generally lateral flow assays and have been developed for detecting antigens such as the SARS-CoV-2 virus and for detecting antibodies (IgM and IgG) against SARS-CoV-2.

Rapid antigen tests would theoretically provide the advantage of fast time to result and low cost of detection but are likely to suffer from poor sensitivities based on previous experiences with flu viruses[22,23,24]. Nonspecific responses of IgM and weeks required to develop specific IgG responses limit their use in active case management but can play a role in diagnosing late infection. A significant perspective to consider is the potential utilization of serology in the asymptomatic population or those presenting with mild symptoms (including health care workers). These are a substantial group of people who are not tested for viral RNA (for practical reasons) thereby masking a population's true rate of infection. As the epidemic progresses and more large-scale studies become available, serology may offer the greatest potential to understand the true scale of human-to-human transmission of the 2019 novel coronavirus.

Currently, there are an increasing number of in vitro diagnostic companies that are developing or have developed tests for antibodies (https://www.finddx.org/covid-19/pipeline/). Five of the seventeen antigen-detection rapid diagnostic tests and 26 of the 53 antibody detection tests reported on the website have been selected for the first round of

evaluation. Additional tests will continue to be reviewed on a rolling basis. Cellix was the first company to get emergency use status from the FDA. Ortho Clinical's VITROS and Mesa Biotech's Accula SARS-CoV-2 tests are the others that got this approval.

2.4. *Nucleic acid testing or molecular testing*

Deep sequencing molecular methods such as the next-generation sequencing and metagenomic next-generation sequencing will play an important role in determining future SARS-CoV-2 mutation but are currently of no use in the diagnosis of COVID-19. Cases of SARS-CoV-2 tested negative for endemic HCoVs included in the molecular repository panels.[9] Thereby indicating the need for different sets of target oligonucleotides.

Eleven molecular devices for diagnosis of SARS-CoV-2 had received urgent approval from National Medical Products Administration (NMPA) during the growing outbreak in China. Variable performance has been reported on these devices.[25–28] It is however important to note that nine out of these eleven devices incorporated real-time RT-PCR techniques with hydrolysis probes. Since then most of the molecular diagnostics being developed for the diagnosis of COVID-19 infections involve real-time RT-PCR. A major advantage of real-time RTPCR assays is that amplification and analysis are done simultaneously in a closed system to minimize false-positive results associated with amplification product contamination. Several RTPCR protocols for detection of SARSCoV-2 RNA have been posted by world health organization (http://www.who.int/emegencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance). Of these, the protocols from US Centers for Disease Control and Prevention (CDC),[29] The Charité Institute of Virology in Berlin, Germany[30] and the Hong Kong University[31] are widely utilized. Other molecular methods that are being evaluated include the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), the Loop-Mediated Isothermal Amplification (LAMP), and multiplex isothermal amplification followed by microarray.[32]

2.5. *Target selection for RT-PCR*

Coronaviruses have a number of molecular targets within their positive-sense, single-stranded RNA genome that can be used for PCR assays.[11,13] These include structural proteins, including envelope glycoproteins spike (S), envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N). In addition to these genes that encode structural proteins, there are species-specific accessory genes that are required for viral replication. These include RNA dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE), and open reading frames 136 ORF1a and ORF1b.[1,9]

To avoid possible cross-reaction with other endemic coronaviruses, at least two molecular targets should be included in the assay for detection of SARS-CoV-2. The ideal design would include at least one conserved region and one specific region to mitigate against the effects of genetic drift, especially as the virus evolves within new populations. The CDC recommends two nucleocapsid targets (N1 and N2) while WHO recommends initial screening with E gene followed by confirmation using the RdRp.[1]

3. Result interpretation

If all the targets in the RT-PCR assay test positive, a case is considered to be laboratory confirmed. A cycle threshold value (Ct-value) less than 40 is defined as a positive test, while a Ct-value of 40 or more is defined as a negative test. A Ct-value <40 only one of the two targets is defined as indeterminant and requires confirmation by retesting.[30] Currently the assays with three targets, positives for two or more targets are considered positive.

4. Future directions

Currently, most of the COVID-19 testing is performed in the laboratory environment. Accurate and scalable point-of-care (POC) tests would increase the scope of testing in the community outside the laboratory settings.[1] Diagnostics developers are now striving to bring fast SARS CoV-2 tests to market as soon as possible. These short-turnaround-time tests will be very important for real-time patient management and infection control decisions. Six commercially available POC tests Xpert SARS-Cov-2 by Cepheid, VitaPCR COVID-19 assay by Credo, RapiPrep COVID-19 by Microsens Dx, ePlex SARS-CoV-2 by GlenMark Diagnostics, Accula SARS-CoV-2 by Mesa Biotech and ID NOW COVID-19 by Abbott Diagnostics have shown promising results and have obtained either the CE marking or the emergency use approval. The time to result of these tests varies from 13 minutes (Abbott ID NOW) to 45 minutes (Cepheid Xpert Xpress). None of these devices, however, have evidence of clinical diagnostic accuracy so far. These tests have been validated on a small number of spiked samples in the laboratory (20–50 positive samples).

The release of new and reliable tests or expanding the capacity of tests will go a long way in curbing the pandemic. To this date more than 100 tests have received emergency use authorization by the US FDA (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations-medical-devices). The choice of the sample is also critical. Although Nasopharyngeal swabs have shown superiority over the oropharyngeal swabs, to increase the yield an oropharyngeal swab can be combined with a nasopharyngeal swab but this would require twice the number of swabs. Alternative samples such as the saliva and nasal washes could be used for epidemiological screening. Recently a test developed by Rutgers, RUCDR Infinite Biologics and its collaborators, Spectrum Solutions and Accurate Diagnostic Labs which use saliva have obtained FDA emergency use approval (https://www.fda.gov/medical-devices/emergency-situations-medicaldevices/emergency-useauthorizations). The role of rectal swabs in managing patients with late infection is currently not well understood and needs more research.

Conflicts of interest

The authors have none to declare.

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