Diagnostic tests for SARS-CoV-2: current status and issues

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Abstract

The current coronavirus disease 2019 (COVID-19) pandemic has affected the whole world. Accurate, rapid and affordable diagnostic testing for COVID-19 is crucial to prevent and control this global pandemic. This paper reviews the current status and issues related to diagnostic tests for COVID-19.

Introduction

The current outbreak of coronavirus disease 2019 (COVID-19) which emerged in Wuhan, China, is caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. World Health Organization (WHO) declared SARS-CoV-2 a pandemic on March 11, 2020 [2]. Accurate, rapid and affordable diagnostic testing for COVID-19 is crucial to prevent and control this global pandemic. The global approach to SARS-CoV-2 testing has been non-uniform. In South Korea, individuals with respiratory illness and any contacts with COVID-19 are tested whereas Spain initially limited testing to individuals with severe symptoms or those at high risk of developing them [3].

The vital role of highly sensitive and specific diagnostic assay in the control of infectious epidemic was evidenced around two decades back, in 2002/2003, when SARS-CoV emerged in Southeast Asia and when MERS-CoV emerged in Middle East back in 2012. The concerted efforts of public health authorities by means of rapid testing of suspected cases interrupted the chain of transmission and helped contain the outbreak. Moreover, valid, rapid, sensitive and specific laboratory diagnostic tools are essential for proper case identification, timely management of the patients, contact tracing, animal source finding, and rationalization of infection control measures in COVID-19 [4].

Several approaches have been used to devise rapid and affordable test(s) to detect COVID-19 cases efficiently and as early as possible to prevent and control this highly transmissible disease. The tests include virus culture, molecular technique for detection of viral nucleic acid and immunoassays.

Virus culture

Diagnosis using viral culture is not useful, as it takes at least 3-6 days for SARS-CoV-2 to cause apparent cytopathic effects in selected cell lines, such as VeroE6 cells. Moreover, isolation of the virus requires highly skilled manpower; expensive equipment and biosafety level 3 facilities, which are not available in most health care institutions [5].

Molecular assays

A real-time RT-PCR (reverse transcriptase polymerase chain reaction) method is recommended for detecting SARS-CoV-2 during the period of viral shedding in acute phase of COVID-19, till date. However, this method, when used alone, has limitation in the detection of the virus during different phases of the illness as the viral load of SARS-CoV-2 is very high when symptoms appear (overall $>1 \times 10^6$ copies/mL) among different clinical specimens [6] and declines

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steadily [7,8]. Another pressing issue regarding using RT-PCR to detect COVID-19 case is the false-negative and false-positive results. Wang et al. reported the failure to diagnose many suspected cases having conventional clinical COVID-19 features including specific computed tomography (CT) images led to inefficient separation of many potential cases and hindered the control strategy [9]. Following infection, SARS-CoV-2 undergoes immunologic pressure in humans and accumulate mutations, which may affect not only its transmissibility and virulence but also its detectability with the same RT-PCR kit overtime [10]. One study detected 93 mutations among 86 complete or near complete genome of SARS-CoV-2 [11]. Similarly, mutations in the primer and probe target regions of the SARS-CoV-2 genome for RT-PCR may produce false-negative results. Although, in order to mitigate this problem, several types of SARS-CoV-2 RT-qPCR kit have been devised targeting the conserved regions of the viral genome and targeting multiple target gene amplification. However, variability resulting in mismatches between the primers and probes and the target sequences might reduce the assay performance. Moreover, the viral load of SARS-CoV-2 in different anatomic sites, sampling timing, sampling procedures and stage of the disease play important roles in producing false-negative results [12].

Specimens are generally collected from both the upper respiratory tract (URT; nasopharynx and oropharynx) and lower respiratory tract (LRT; expectorated sputum, endotracheal aspirate, or bronchoalveolar lavage) for COVID-19 testing by RT-PCR. The virus is also detected in fecal and blood specimens [13]. The sensitivity/positivity rate of RT-PCR in various biological samples of COVID-19 patients is shown in Table-1. The sensitivity/positivity rate varies from 3.03% to 93% in various clinical samples. A study observed strong correlation of viraemia with the disease severity [14]. SARS-CoV-2 quantification in plasma/serum could also represent a potentially useful early diagnostic and prognostic tool [15]. According to Guangzhou CDC, virus can be detected in upper respiratory samples 1-2 days prior to symptom onset and persists for 7-12 days in moderate cases and up to 2 weeks in severe cases [13]. Prolonged viral shedding from nasopharyngeal aspirates – up

to at least 24 days after symptom onset – was reported among COVID-19 patients in Singapore [16]. Viral RNA has been detected in feces in up to 30% of patients from day 5 following onset of symptoms and has been noted for up to 4-5 weeks in moderate cases [13]. Fang et al. found first RT-PCR test positive in 71% cases after studied on first throat swab or sputum samples from 51 patients. In second RT-PCR, another 23% cases became positive who were initially negative. In third and fourth RT-PCR, another 4% and 2% cases became positive [17]. Another study conducting serial RT-PCR testing showed the mean time from an initial negative RT-PCR to subsequent positive RT-PCR was 5.1 days (± 1.5 days) [18]. Regarding asymptomatic patients, Arons et al. reported that more than half of subjects with positive test results were asymptomatic at the time of testing [19]. Zou et al. reported that the viral load of asymptomatic patients was similar to symptomatic patients, indicating a transmission potential of asymptomatic or pre-symptomatic patients. The study reported that patients with few or no symptoms had modest levels of detectable viral RNA in the oropharynx for at least 5 days [20].

Table-1: *The sensitivity/positivity rate of RT-PCR in different specimens during acute phase of COVID-19 patients*

Specimen	Sensitivity/ positivity rate (%)	References
Nasopharyngeal swab	63 – 89	[21,22]
Oropharyngeal swab	32 – 84	[21,23]
Brochoalveolar lavage	93	[21]
Sputum	72	[21]
Blood	3.03 – 15	[24,25]
Feces	9.83	[24]
Anal swab	10-21.2	[24,26]

RT-PCR assay targets the open reading frames (ORF1a and ORF1b), non-structural protein (nsp14), RNA-dependent RNA polymerase (RdRp), envelope glycoproteins spike (S), envelope (E), nucleocapsid (N), or helicase (Hel) gene of SARS-CoV-2. To avoid potential cross-reaction with other endemic coronaviruses as well as potential genetic drift of

SARS-CoV-2, at least two molecular targets should be included in the assay. Various investigators in different countries have used a number of these molecular targets for real-time RT-PCR assays [27]. In the United States, the US Centers for Disease Control and Prevention (CDC) recommends two nucleocapsid protein targets (N1 and N2) [28] while WHO recommends first line screening with the E gene assay followed by a confirmatory assay using the RdRp gene [29]. Another study in Hong Kong, China used two targets for their RT-PCR assay; the first used the nucleocapsid for screening followed by confirmation by the open reading frame 1b [30]. Chan et al. developed and compared the performance of three novel real-time RT-PCR assays targeting the RdRp/Hel, S, and N genes of SARS-CoV-2. Among them, the COVID-19-RdRp/Hel assay had the lowest limit of detection *in vitro* and higher sensitivity and specificity [31]. The analytical sensitivity of different RT-PCR test kits varies from 0.15 to 100 copy/µL [32].The US CDC recommends that negative results of real time RT-PCR testing for SARS-CoV-2 from at least two sequential respiratory tract specimens collected at least 24 hours apart can be considered to discontinue transmission-based precautions [33].

Immunoassays

Success of PCR-based diagnostics relies on timing and technique of sampling, stage of the infection, type of sample, the kinetics of viraemia and shedding of virus throughout the course of infection. Moreover, inadequate access to reagents, expensive equipment and bio-safety facilities have resulted in low efficiency in handling large number of samples in-time delivery of reports. Therefore, serological testing is crucial to complement the RT-qPCR. In addition, serology is used as an important tool to monitor the evolution of an outbreak, retrospective studies of asymptomatic and mild cases and animal reservoir identification [34,35]. However, devising serologic assays targeting immunogenic proteins is difficult because closely related viruses may share common epitopes that elicit cross-reactive and cross-neutralizing antibodies. Within a genus, antibodies against other coronaviruses might cross-react and such cross-reactive conserved viral proteins limit

the use of whole virus–based assays, for example, immunofluorescence assay (IFA) [7]. Also whole virus based assays primarily require viral culture which is difficult to establish.

Several immunoassays have been developed for rapid detection of SARS-CoV-2 antigens or antibodies to overcome this hurdle. Immunoassays tests include rapid lateral flow assays, ELISA and chemiluminescence. These serological tests provide the advantage of fast and low-cost detection of SARS-CoV-2 but are likely to suffer from poor sensitivity during acute phase of the disease [27].

According to recent studies, serological testing identifies convalescent cases or people with milder disease or patients who present late with a very low viral load, below the detection limit of RT-PCR assays. One study evaluated two recombinant SARS-CoV-2 nucleocapsid protein (rN) and spike protein (rS) based ELISA kits for detection of IgM and IgG antibodies. They found high sensitivity in samples collected from patients 10 days post-disease onset. They observed that IgM and IgG positivity rate increased with increasing interval of days following onset of disease [1]. Serum IgG was found to rise at the same time or earlier than those of IgM against SARS-CoV-2. It was reported that a higher proportion of patients had earlier IgG than IgM seroconversion probably due to lower sensitivity of the IgM ELISA [8]. CDC's serologic test designed to detect antibodies against SARS-CoV-2 spike protein antigen has a specificity of greater than 99% and a sensitivity of 96%. It can be used to identify past SARS-CoV-2 infection in people who were infected at least 1 to 3 weeks previously [36]. Different antibody detection assays have been approved in different countries for diagnostic and/or research use. The sensitivity and specificity of those methods are shown in Table-2. Several antigens mainly spike and nucleocapsid proteins have been used as capture-antigen in ELISA and lateral flow assay/rapid diagnostic test (RDT) to diagnose IgG and/or IgM against SARS-CoV-2 [37]. Nevertheless, cross-reactivity of antibodies to closely related viruses is a potential issue to interpret the serological test results.

A South Korean antigen detection kit reported 84.38% and 100% sensitivity and specificity respectively using nasopharyngeal swabs from 202

symptomatic patients for detection of SARS-CoV-2 antigen [38]. Quidel Sofia, USA SARS antigen test kit has been reported to detect antigen targeting nucleocapsid protein from SARS-CoV-2 with 96.7% sensitivity within five days of the onset of symptoms. The kit detects viral antigen in nasopharyngeal or nasal swab using immunofluorescence-based lateral flow technology [39]. Another antigen detection kit developed in Japan that detects neucleocapsid protein antigen of SARS-CoV-2 in nasopharyngeal swab using immunochromatographic assay has almost similar sensitivity and specificity [40].

Table-2: *Sensitivity and specificity of antibody detection assays for COVID-19 [37]*

Test format	Antibody	Sensitivity (%)	Specificity (%)
ELISA	IgG	>90–100	>95–100
	IgM	>95	>95
RDT	IgG and IgM	82–93.8	95.6–100

Note: ELISA=enzyme linked immunosorbent assay; RDT= rapid diagnostic assay.

Transformation of the script of laboratory based diagnostic approach into a self-conducted, non-invasive, rapid, convenient, cheap and available over-the-counter diagnostic test, be it less sensitive than the current RT-PCR or serology assay, would enable mass people detect themselves as suspected cases. Thus they could self-isolate far ahead of time than if they were to be diagnosed by the conventional laboratory tests. As long as these people stay home, it would provide a kind of artificial herd immunity which will interrupt the chain of transmission to impede the pandemic. Moreover, these suspected cases would be able to confirm the infection status later by the more specific laboratory tests. The most important issue is that, this strategy would best utilize the minimal resources and the narrow window of time that is not achievable with more sensitive but expensive and time consuming PCR tests. Sherlock Biosciences of Harvard's Wyss Institute for Biologically Inspired Engineering and E25Bio-rooting from Massachusetts Institute of Technology (MIT) and Harvard have developed an inexpensive

paper-based test which can be conducted at home with saliva or nasal mucous, just like doing at-home pregnancy test [41].

Symptom-based diagnosis

A simple and technology independent diagnostic tool, if available, would be immensely valuable to handle the present COVID-19 pandemic. Historically, after initial detection and confirmation of the offending microbe, many previous epidemics were combated without laboratory testing of every case. Subsequently, the cases were detected and managed by typical clinical features of the disease. Therefore, clinical symptom and sign based diagnostic approach may also be a valuable and useful instrument to diagnose COVID-19 in places where RT-PCR or other serological tests are not easily available. There is paucity of studies with regard to the sensitivity and specificity of such symptom based diagnosis of COVID-19. Few studies that considered symptoms based diagnosis of COVID-19 assessed symptoms alone. A Cochrane systematic review reported that till the end of April 2020 no study assessed combinations of different signs and symptoms to diagnose a case of COVID-19. The review revealed a sensitivity of 50% and specificity of 90% when six symptoms (cough, sore throat, fever, myalgia or arthralgia, fatigue, and headache) were considered [42]. The symptom of "sudden smell loss" has been associated with 97% specificity and a sensitivity of 65% with positive and negative predictive values of 63% and 97% respectively for COVID-19 [43].

Therefore, combined symptoms and signs (may be including imaging characteristics) based diagnostic tool for COVID-19 should be developed and tested for sensitivity and specificity in different areas and healthcare settings. Such technology independent tool might help in primary care, emergency or in telemedicine services in resource poor countries/regions to identify COVID-19 patients at low cost and thus would minimize its spread. Moreover, it would reduce the cost of diagnosing COVID-19 even in hospitals with all facilities by avoiding expensive RT-PCR test in every case.

Conclusion

The pandemic of SARS-CoV-2 infection has emphasized the importance of simple, fast and affordable high quality diagnostic tools to limit the spread as well as to appropriately treat the COVID-19 patients. Further studies are needed to develop easy to use assays of similar sensitivity and specificity of RT-PCR. Symptom/sign based technology independent diagnostic tool for detection of COVID-19 deserves further attention because that can be used at all levels of healthcare facilities.

Acknowledgement

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