COVID-19 diagnostic multiplicity and its role in community surveillance and control

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INTRODUCTION
Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the seventh coronavirus that has crossed the species barrier and has emerged as a global health emergency [1]. The first case of coronavirus disease (COVID-19) was reported in December 2019 at Wuhan, Hubei Province, China [2]. On 11th March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic [3]. There were only 11953 cases of COVID-19 with 259 reported deaths till 1st Feb 2020. This has exponentially increased to more than 3 million cases with 0.2 million deaths as of 30th April 2020 (Figure 1) [4].

SARS-CoV-2 is an enveloped positive single stranded RNA virus belonging to Betacoronavirus genus, of Orthocoronavirinae subfamily in the Coronavirus family of order Nidovirales [5]. Like other betacoronaviruses, SARS-CoV-2 has Spike glycoprotein (S), Matrix proteins (M) and outer envelope (E) encapsulating the RNA and nucleoprotein (N) (Figure 2). Apart from these, the viral genome also encodes for proteins like RNA dependent RNA polymerase (RdRp) and 6 accessory ORF1ab, ORF3a, ORF6, ORF7a, ORF7b, and ORF8 proteins [6]. Genomic analysis has shown that SARS-CoV-2 has 79.6% sequence identity to SARS-CoV and 96% identity with bat coronavirus (BatCoV RaTG13)
The virus entry is via the respiratory route where S protein mediates viral binding onto cells expressing ACE2 (angiotensin converting enzyme 2) receptor. Cellular serine protease TMPRSS2 present on the host cell is used by SARS-CoV-2 for S protein priming [8, 9]. After receptor mediated endocytosis the viral genome is released in the cytosol that translates replicase polyproteins. These polyproteins subsequently get cleaved and further assemble to form replicase transcriptase complex to help in RNA replication and sub-genomic RNA transcription SARS-CoV-2 has evolved into 2 strains designated as L and S strains [10]. L strain is more aggressive and was prevalent during early stages of the epidemic in Wuhan [11]. Screening is our window into the pandemic and its spread. Diagnosis of persons exposed to/infected with SARS–CoV-2 is central to controlling
the global pandemic of COVID-19. Few countries have upscaled diagnostic testing on a massive scale to successfully contain the spread of the pandemic. In contrast, poor resource countries like India have prioritized testing for specific groups of persons. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) based assays are considered the reference standard for COVID-19 diagnostics. But the test protocol is complex and expensive, however, and is mainly suited to large, centralized diagnostic laboratories. This has inhibited upscaling of testing capacity. To overcome this barrier, point-of-care technologies and serologic immunoassays are rapidly emerging. But the performance of these have not been evaluated adequately. These challenges are even greater in low-resource settings. Currently, several diagnostic modalities (Clinical, molecular, immune-based and digital) are available for COVID-19, each with its own pros and cons (Figure 3). Although, there is a global consensus to increase the testing capacity, it is also essential to prudently utilize these tests to control the pandemic. In the current scenario of information overload in the field of COVID-19 diagnostics, we have reviewed the current array of diagnostics for SARS-CoV-2, highlighted gaps in current diagnostic modalities, and their role in community surveillance and control of the pandemic.

Different Modalities of COVID-19 Diagnostics

Clinical and radiological diagnosis of COVID-19

COVID-19 presents with 3 clinical stages of infection after incubation period of 2-14 days. Stage 1 - asymptomatic, Stage 2 - Upper airway and conducting airway response and Stage 3 - Hypoxia, ground glass infiltrates, and progression to Acute Respiratory Distress Syndrome (ARDS) [12]. The stages and severity varies depending on the age, immune status of the individuals and associated co-morbidities [13]. High viral load can be an important marker for severity of the disease and such patients also have long virus shredding period [14].

Figure 3 - Various COVID-19 diagnostic modalities.
The clinical diagnosis of SARS-CoV-2 is fever, dry cough and shortness of breath and may lead to severe form such as respiratory distress and failure [15]. Respiratory failure that necessitates mechanical ventilation and support in an intensive care unit (ICU), can further cause multi-organ and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes. A case study by Li et al shows that the mean age of suffering from COVID-19 was around 59 years ranging from 15 to 89 years [16]. Patients with comorbidities (cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancers) had higher case-fatality rates (10.5%, 7.3%, 6.5%, 6.0%, and 5.6%, respectively) than those without comorbidities (0.9%) [17]. Based on the presentation of symptoms and respiratory parameters, disease severity is divided into mild to moderate, severe and critical.

- Mild disease: non-pneumonia and mild pneumonia; this occurred in 81% of cases.
- Severe disease: dyspnea, respiratory frequency ≥30 min, blood oxygen saturation (SpO2) ≤93%, PaO2/FiO2 ratio or P/F [the ratio between the blood pressure of the oxygen (partial pressure of oxygen, PaO2) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO2)] <300, and/or lung infiltrates >50% within 24 to 48 hours; this occurred in 14% of cases.
- Critical disease: respiratory failure, septic shock, and/or multiple organ dysfunction or failure; this occurred in 5% of cases.

CDC has added six new symptoms to its list for COVID-19: chills, muscle pain, headache, sore throat, repeated shaking with chills and a loss of taste or smell [18]. Kaye et al. reported anosmia in 73% of patients prior to COVID-19 diagnosis and was initial symptom in 26.6% of patients [19].

COVID-19 infection causes a severe lower respiratory tract infection with bilateral, basal and peripheral predominant ground-glass opacity, consolidation or both as the most common reported chest radiological findings. These findings peak around 9-13 days and slowly begin to resolve thereafter [20].

**Laboratory based Molecular Diagnostics**

Laboratory based molecular diagnostics are the hallmark of diagnosis of COVID-19. Currently, the diagnosis of COVID-19 is based on testing

The nasopharyngeal or oropharyngeal samples collected from suspected patients. RT-PCR based tests are the standard reference for diagnosis of COVID-19. A study by Wang et al. showed higher positivity in nasopharyngeal swabs than oropharyngeal swabs, especially among hospitalized patients [21]. A nasopharyngeal swab is the preferred choice for swab-based SARS-CoV-2 testing but sometimes oropharyngeal, mid-turbinate and anterior nares samples are also tested. A study by Wu J et al. found that positivity of SARS-CoV-2 nucleic acid in the sputum of 132 patients with COVID-19 was higher than that of nasopharyngeal swabs, and viral nucleic acids were also detected in blood and digestive tract (faecal/anal swabs) [22]. Detection of SARS-CoV-2 nucleic acid in nasopharyngeal swab alone does not yield high positivity, multi-sample SARS-CoV-2 nucleic acid detection can improve the accuracy, reduce false-negative rate and better guide clinical treatment [22]. Samples should be collected using flocked swabs to increase the collection of viral load and release of cellular material. Certain specific swabs are not used for the collection of viral loaded samples such as those containing calcium alginate, wood or cotton because they contain material that inhibits PCR assays.

RT-PCR is capable of providing relatively fast results through amplification of low viral RNA with high sensitivity and specificity. The oligonucleotide primers and probes for SARS-CoV-2 detection are usually derived from RNA-dependent RNA polymerase (RdRp) gene in open reading frame (ORF), nucleocapsid (N), envelope (E) regions of the virus [23]. RT-PCR assay can be either a one-step or a two-step assay. In a one-step assay, conversion of RNA to cDNA and further PCR amplification are performed in single reaction tube. Although, this assay provides quick and reproducible results, optimizing the protocol is a challenging step. In contrast, the two-step assay is carried out sequentially in two separate tubes. In comparison to one step PCR assay, this format is more sensitive, but time-consuming [24, 25].

Limited evidence suggests that the viral load peaks during the first week of illness, then gradually declines over the second week [26]. Viral presence has also been noted in some patients 28 days after onset of symptoms. High viral load during the early phase of illness suggests that patients could be most infectious during this period,
and this might account for the high transmissibility of SARS-CoV-2.

Though, RT-PCR provides a highly sensitive and specific method for detection of infectious diseases, these methods are typically restricted in a specialized clinical laboratory and are not suitable for quick, easy, point of care diagnostic applications. Currently, reverse transcription loop-mediated isothermal amplification (RT-LAMP) is in development and testing phase for SARS-CoV-2 detection [27]. This highly specific technique uses DNA polymerase and specially designed primers that recognize distinct target sequences on the target genome. In general, there are two inner primers and two outer primers designed to synthesize new DNA strands [28]. The reaction occurs in less than an hour under isothermal conditions at 60-65°C. The approach is much more efficient while still obtaining a high level of precision, less background signal, convenient visualization for detection and does not need sophisticated equipment [28].

CRISPR-based detection can also provide a rapid, highly sensitive and specific approach for molecular based diagnostics. CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique for the detection of COV-ID-19 uses a variant of Cas9 called Cas13 that gets activated by binding to SARS-CoV-2-specific guide RNA [29]. Detection is through fluorescent signal produced by Cas13 mediated cleavage of fluorophore-quencher probes. Another CRISPR-based DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay uses Cas 12a to provide a faster alternative to real-time RT–PCR assay [30].

There are several other additional novel diagnostic methods in developmental phase or in evaluation. The Foundation for Innovative New Diagnostics (FIND) is conducting independent evaluations of molecular tests and immunoassays available for COVID-19 diagnostics, in collaboration with the WHO, the University Hospitals of Geneva (HUG) and others (Supplementary Table 1 and 2). Results for the first round of independent evaluation of COVID-19 PCR Based tests has been released and depicted in Table 1.

### Table 1 - Evaluation of COVID-19 PCR based test.

<table>
<thead>
<tr>
<th>Company</th>
<th>Gene target</th>
<th>Copies / reaction</th>
<th>Avg Ct value</th>
<th>Clinical sensitivity</th>
<th>Clinical specificity</th>
<th>Supplier recommended Ct cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altona Diagnostics</td>
<td>E</td>
<td>1–10</td>
<td>35.45</td>
<td>92%</td>
<td>100%</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>1–10</td>
<td>35.99</td>
<td>92%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>BGI Health (HK) Co. Ltd</td>
<td>ORF1</td>
<td>1–10</td>
<td>32.43</td>
<td>100%</td>
<td>99%</td>
<td>≤38</td>
</tr>
<tr>
<td>Boditech Med. Inc.</td>
<td>E</td>
<td>10–50</td>
<td>34.9</td>
<td>100%</td>
<td>100%</td>
<td>≤42</td>
</tr>
<tr>
<td></td>
<td>RdRP</td>
<td>50–100</td>
<td>33.46</td>
<td>90%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>DAAN Gene Co. Ltd</td>
<td>ORF1</td>
<td>1–10</td>
<td>38.76</td>
<td>100%</td>
<td>96%</td>
<td>≤40</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1–10</td>
<td>36.97</td>
<td>100%</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td>GeneFirst Limited</td>
<td>ORF1</td>
<td>1–10</td>
<td>35.45</td>
<td>100%</td>
<td>99%</td>
<td>≤37</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1–10</td>
<td>36.72</td>
<td>98%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>KH Medical Co. Ltd</td>
<td>S</td>
<td>1–10</td>
<td>37.94</td>
<td>100%</td>
<td>100%</td>
<td>≤40</td>
</tr>
<tr>
<td></td>
<td>RdRP</td>
<td>10–50</td>
<td>36.74</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>SD Biosensor Inc.</td>
<td>E</td>
<td>1–10</td>
<td>37.43</td>
<td>100%</td>
<td>97%</td>
<td>≤41</td>
</tr>
<tr>
<td></td>
<td>ORF1</td>
<td>1–10</td>
<td>36.99</td>
<td>100%</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td>Tib Molbiol</td>
<td>E</td>
<td>1–10</td>
<td>33.34</td>
<td>100%</td>
<td>100%</td>
<td>&gt;2-4 cycle higher than Ct value of 10 copies</td>
</tr>
</tbody>
</table>
BioFire FilmArray (bioMérieux), Cobas Liat (Roche Diagnostics), and GeneXpert (Cepheid) [31]. The Xpert Xpress SARS-CoV-2 test (Cepheid) (FDA Emergency Use Authorization) utilizes the GeneXpert platform, which is widely used for tuberculosis and HIV testing, especially in low- and middle-income countries. This capacity might be useful to scale up testing across the world, especially in resource poor settings.

**Antigen detection tests**

One type of RDT detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a respiratory sample. If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip and generate a visually detectable signal, typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, such tests are recommended to identify acute or early infection. The performance of these tests depends on the time from onset of illness, the concentration of virus in the specimen, the quality of the specimen collected from a person and how it is processed. Other antigen-based RDTs for other respiratory viruses such as influenza have demonstrated the sensitivity of these tests to vary from 34% to 80% [32]. Based on this information, half or more of COVID-19 infected patients might be missed by such tests. With the limited data now available, WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for clinical decision making, although research into their performance and potential diagnostic utility is highly encouraged.

According to Seo G et al., field-effect transistor (FET)-based biosensing device for detecting SARS-CoV-2 can be used in clinical samples [33]. The sensor was fabricated by coating graphene sheets of the FET with a precise antibody against SARS-CoV-2 spike protein. The functioning of the sensor was determined using antigen protein, cultured virus, and nasopharyngeal swab specimens from COVID-19 patients. The FET device could sense the SARS-CoV-2 spike protein at concentrations of 1 fg/mL in phosphate-buffered saline and 100 fg/mL clinical transport medium [34]. Monoclonal antibodies against the nucleocapsid protein of SARS-CoV-2 have also been generated, which might form the basis of a future rapid antigen detection test [35].

**Antibody detection tests**

It is a known fact that identification of IgM/IgG antibodies is a much less complex process than molecular identification of virus [36]. The assays can be performed on the samples collected from blood or saliva. The “serological” tests which rely on detection of antibodies are usually against the nucleocapsid or spike proteins in the sample. A negative result in the serological assays will not assure the absence of infection. Sometimes, cross-reactivity of the non-SARS-CoV-2 coronavirus protein is also a potential problem [37]. These IgM/IgG detection assays are more reliable in conditions where patients present to the hospital in the late stage of infection, when RT-PCR may be falsely negative due to decrease in the viral shedding [38].

After SARS infection, IgM antibody could be detected in patient’s sample after 3-6 days and IgG after 8 days [39]. However, the antibody response to SARS-CoV-2 has shown different profile as per limited serological studies. IgM and IgG appear 2-4 after the onset of symptoms with the median number of days for seroconversion being 10-13 days. Detection of IgM against SARS-CoV-2 tends to indicate recent exposure, whereas the detection of IgG indicates prolonged exposure to the virus. The detection of both IgM and IgG could provide useful information on the virus infection time course. These antibody kits could be IgM, IgG or combined IgM/IgG detection kits.

Apart from these rapid kits, many ELISA based antigen or antibody kits have been approved for diagnostic or research purpose, with several others in the process of development (Supplementary Table 1 and 2). Unlike rapid test kits, ELISA provide quantification of antibodies and are less vulnerable to false-positive and false-negative reactions.

**Digital diagnostics**

In this era of machine learning, digital diagnostics has come up as a new innovation in medical field as a complimentary tool for standard screening and diagnostic tests. Current COVID-19 outbreak provided another opportunity for Artificial Intelligence (AI) application to prove it’s worth in health care settings. Two such examples are
Infervision and Intrasense Myrian, which are algorithm based AI technologies developed to read clinical images [40, 41]. These algorithms distinguish between lung lesions of COVID-19 and other respiratory infections. They basically measure volume, shape and density and compare changes of multiple lung lesions from an image to provide quantitative report in order to assist healthcare workers make quick decisions. Another, AI-based deep learning structure COVID-diagnosis-Net, showed a high accuracy of 98.3% in processing and analysing X-ray image for the early stage detections of the COVID-19 cases [42].

Another digital diagnostic tool which is in development is AiroStotleCV19, a breath test for volatile organic compounds (VOCs). Being a viral infection, COVID-19 induces oxidative stress. Developers are working on the identification of oxidative stress biomarkers during breath test for early diagnosis COVID-19.

Digital technologies are highly sensitive, specific, non-invasive and cost-effective. They can help in reducing the timeframe and workload needed in dealing with high number of cases, hence minimizing the risk of transmission to other patients and hospital staff [43].

Community surveillance and control

Being resource intensive and costly, current molecular based tests are used for confirmation of COVID-19 among possible suspects, most often the symptomatic patients. However, apart from transmission from symptomatic patients, pre-symptomatic and asymptomatic transmission plays a key role in driving disease transmission across communities, especially due to the hidden nature of the spread.

Pre-symptomatic transmission: The incubation period for COVID-19 is around 5-6 days, lasting up to 14 days. During this period, also known as the pre-symptomatic period, people can be contagious and transmission can occur. Pre-symptomatic transmission has been documented through contact tracing efforts and enhanced investigation of clusters of confirmed cases [44-46]. Data suggests that some people can test positive for COVID-19 from 1-3 days before they develop symptoms which makes it more likely that people infected with COVID-19 could transmit the virus before significant symptoms develop [45].

Asymptomatic transmission: An asymptomatic laboratory-confirmed case is a person infected with COVID-19 who does not develop symptoms. Asymptomatic transmission refers to transmission of the virus from a person, who does not develop symptoms. A recent study in NEJM reported that a viral load detected in an asymptomatic patient was similar to that detected in symptomatic patients, indicating the potential for transmission in asymptomatic patients [47]. On January 24, The Lancet reported a familial cluster of SARS-CoV-2 infection with a travel history to Wuhan, with their asymptomatic child presenting with no fever, respiratory tract symptoms or diarrhoea but had ground-glass lung opacities seen on radiography [48]. Subsequently, several asymptomatic patients were confirmed to have COVID-19 in many Chinese cities with most of them having an epidemiological history with a potential of infecting others.

Early detection and isolation of these hidden cases is necessary to reduce the size of the outbreak of SARS-CoV-2. Current strategies have focused on identifying COVID-19 suspect/symptomatic, testing and isolating them. However, we are missing out on asymptomatic transmission that is a major driver of community transmission of the corona virus accounting as high as 80% of transmission. Widespread testing of populations can play a key role in identifying asymptomatic people and isolating them, thus, curbing further transmission. Countries such as South Korea have successfully controlled the pandemic by testing aggressively to identify possible carriers of infection and isolating them effectively (Figure 4). However, in resource-poor settings, where up-scaling of conventional RT-PCR is cumbersome, use of rapid test kits can be a feasible option for population-wide testing.

Rapid diagnostic tests (RDTs) are simple stand-alone antigen/antibody detection tests that can be used at the point of care outside the laboratory/hospital by minimally trained staff and can provide test results within 15 minutes. They are attractive for decentralized testing particularly in low resource settings. These rapid tests can be used to broaden the criteria for testing and include asymptomatics with probable exposure to the virus. In India, RDTs have been approved
for use in hotspots/cluster containment zones to identify asymptomatic persons exposed to the virus and isolating them to prevent further community transmission. However, because of its low specificity, RDT negatives are further confirmed by RT-PCR.

The use of rapid antibody tests is manifold. RDTs could be used in seroprevalence surveys to understand the dynamics of spread of the virus in the community, assess attack rates and extent of an outbreak. It can verify the immune response to vaccines during clinical trials, or be used in contact tracing weeks or longer after a suspected infection, help inform public policy makers about the burden of asymptomatic cases in a population. This is useful for the purpose of community surveillance and understanding the epidemiology of COVID-19 in the country.

A positive test result in the convalescent phase indicates that they will be safe from another infection for at least some time which mean they could return to work or work as a shield for the vulnerable population till we achieve herd immunity. However, there is no evidence that people who have recovered from COVID-19 and have antibodies are protected from a second infection. There have already been some reported cases of re-infection with corona virus.

CONCLUSIONS

Rapid and early detection of the SARS-CoV-2 virus is key to prevent the spread of the virus and control the pandemic. The first line of defence against any outbreak is always developing the diagnostic assays for identification of confirmed
cases and isolating them. Immunoassays against the antigen or antibodies provide the second line of diagnostics and complement nucleic acid tests.

Worldwide lockdown with strict social distancing and use of masks was adopted by most countries to curtail the spread of COVID-19. However, not doubting the efficiency of lockdown, there are high chances of secondary waves of epidemic following the end of this lockdown. Thus, prompt and reliable diagnostic facilities along with appropriate non-pharmacological interventions and vaccines is the need of the hour. The future development of portable assays such as isothermal amplification, barcoding and microfluidic technologies and application of artificial intelligence algorithms could enable point-of-care testing and multiplex assays to be rapidly implemented in an outbreak situation. This approach can reduce mortality and help in curtailing the spread of zoonotic pathogens.

Authors contributions
SCT and VD were involved in conception of the idea, literature review, drafting the manuscript, editing and finalization of the manuscript. JPT was involved in conception of the idea, literature review, critical review, editing and finalization of the manuscript. AP was involved in literature review, critical review of the manuscript and finalization of the manuscript. All the authors gave their approval to the final submitted version of the manuscript.

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REFERENCES

