Review Article

Laboratory Diagnostics in COVID-19: What We Know So Far

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Abstract

COVID-19 took the world by storm in early 2020 which rapidly escalated to a pandemic of unprecedented proportions. Containment of the outbreak requires a robust public health system of surveillance, contact tracing and laboratory preparedness. Technological advancement in molecular diagnosticshas helped identify patients in the initial stages of the outbreak. RT-PCR remains the gold standard in COVID-19 testing. However, as the pandemic continues, there is need for rapid and point of care tests (POCT) for mass screening and rapid decision making. Current serological tests and POCT have high sensitivity but need to be interpreted with caution. This review aims to discuss current laboratory tests available for the diagnosis of COVID-19.

Keywords: COVID-19; SARS-CoV-2;laboratory diagnostics; POCT

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Introduction

Wuhan city in China became the focus of the world in December 2019 when they reported an outbreak of pneumonia of unknown cause. Chinese scientists quickly attributed the outbreak to a novel coronavirus (SARS-CoV-2) later designated by the World Health Organisation (WHO) as COVID-19. The infection quickly spread to the rest of the world and was declared a Public Health Emergency of International Concern on the 30th January 2020 by the WHO. As of writing, there have been over 9.8 million confirmed cases of COVID-19, including nearly 500,000 deaths globally, reported to WHO.

Respiratory viruses have the tendency to escalate into outbreaks if left unchecked due to the nature of the virus itself and the transmission potential of respiratory infections. Over the last century, the world has faced numerous respiratory virus outbreaks. In tackling these outbreaks, we have learned the importance of prompt disease detection, a robust surveillance mechanism and laboratory preparedness [1]. To contain further spread of the virus, WHO director-general Dr Tedros Adhanom Ghebreyesus has urgently called for the increase in capability for testing COVID-19 [2]. Therein lies the importance of diagnostic laboratories. Laboratory space, well-trained staff, user-friendly validated laboratory methods are important elements that need to be considered while choosing the appropriate test kit. Detection of nucleic acid or RNA is the most frequent method used for the identification of COVID-19 among suspected patients and screening for the close contacts. RNA detection is extremely sensitive due to the power of nucleic acid amplification and highly specific by using complementary nucleic acid probes/ primers for the identification of a particular RNA.This review will discuss on current available molecular and serological diagnostic tests including rapid point

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of care testing used for COVID-19 diagnosis. In addition to that, we will also summarize the current testing strategies that utilizes laboratory diagnostics in containing further spread of COVID-19.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR): Gold Standard in COVID-19 Testing

Real-time reverse transcription polymerase chain reaction (RT-PCR) is the current gold standard for detecting SARS-CoV-2. The test is considered highly specific as it is able to reliably detect SARS-CoV-2 from an array of other human respiratory viruses [3]. The speed and breadth at how the pandemic have spread requires a laboratory test that is able to accurately detect SARS-CoV-2 at the early stages of infection when virus copies are low. Generally, real time RT-PCR is not only able to detect viruses but also quantitate the viral load which is valuable in aiding clinicians on patient treatment and predicting disease severity.

The ORF1ab, RdRp, E, N, and S genes are the targets most frequently used for SARS-CoV-2 detection by RT-PCR. Current protocols use a combination of gene targets to accurately diagnose COVID-19[4]. The Charité protocol targets the E gene to screen for sarbecovirus and RdRp as the report found that it was the most sensitive to detect SARS-CoV-2[3].Centre for Disease Control (CDC) recommends detection of 2 types of nucleoproteins specific for SARS-CoV-2[5].

Specimens that can be tested using RT-PCR include the usual respiratory specimens (nasal, pharyngeal, sputum, bronchoalvelolar lavage (BAL) and nasopharyngeal swabs) and also other samples such as urine, blood, stool and anal swabs [6]. It has been observed that higher viral loads were found in nasopharyngeal specimens compared to throat swabs. Thus the preference of nasopharyngeal swabs for screening of SARS-CoV-2 as it more sensitive and less invasive [7–9]. Lower respiratory specimens such as bronchoalveolar lavage (BAL) and sputum were better in detecting COVID as they yielded more positive rates [6].Although realistically, BAL fluid can only be collected in the sickest of patients and sputum can only be collected in patients with pneumonia. The significance of virus detected in urine and stool has yet to be determined. There have been reports where virus detection in blood is an indicator of severe disease suggesting poor prognosis in patients with disseminated viraemia[10,11]. However, researchers have yet to find a connection between a positive SARS-CoV-2 RT-PCR in other specimens and the viral load in predicting disease severity.

RT-PCR allows us to study the viral load kinetics of SARS-CoV-2 giving us insights to the transmissibility and infectious period of this novel virus. The higher viral loads found in upper respiratory specimens may help explain the speed at which the virus has spread.In fact, viral loads in asymptomatic patients was similar to that in mildly symptomatic and symptomatic patients[7,12]. It has been observed a viral load that peaked just before and on symptom onset [7,13]. This is further correlated with a study following close contacts of COVID-19 patients. Based on this, we can conclude that high transmissibility occurs during the first week before and after symptom onset [14]. And even asymptomatic or minimally symptomatic patients have the potential to transmit the virus [7]. This lends us valuable information for public health surveillance of patients and close contacts.A study from Wuhan, the original epicenter of the pandemic, reported median detection of virus by RT-PCR is at 20 days and up to 37 days for COVID-19 survivors [15]. However, there have been reports that viral RNA can still be detected up to 70 days of hospitalization [16]. Even so, detection of virus RNA does not necessarily indicate active disease. Wölfel et al found that isolation of live virus was unsuccessful after the eighth day of symptom onset and cannot be isolated at all from stool samples [17]. This signifies that although positive SARS-COV-2 RT-PCR results persists more than the standard 10 to 14-day quarantine period, live replicating viruses and effectively infectiousness declines after the first week of symptom onset.

To cater for the global need for diagnostic kits, there has been an explosion in development of commercial kits that have not been reliably evaluated.Although SARS-CoV-2 RT-PCR tests were found to be reliably specific, sensitivity of the test varies across different commercial kits. Recent evidence has shown that the rate of false negative tested with RT-PCR is higher than expected. Independent evaluation of several available commercial RT-PCR kits showed wide differences in sensitivity, limit of detection (LOD) and Ct values [18]. There have been reports where initial RT-PCR results were negative but became positive after repeated testing[8,19]. Kim et al studied the viral load kinetics of SARS-CoV-2 in different patients. It was proposed that the timing of sampling and the stage of disease development influences real-time RT-PCR results [20]. Interestingly, several reports have emerged of varied results from RT-PCR tests at different time points but which came from the same patients within the same hospitalisation[21,22]. This points to

issues regarding the integrity of the sample from the method of collecting the sample to transportation and optimization of RT-PCR.Although it was seen that repeated testing coupled with chest CT improved the sensitivity of diagnosing COVID-19[7,19].Given the propensity to rely on laboratory tests to aid in the diagnosis and management of COVID-19 patients, the high incidence of false negatives is worrying.

Although RT-PCR allows laboratories to perform tests on a relatively fast turnaround time and at a large scale, there is still urgent need for rapid and point-of-care testing (POCT). RT-PCR generally yields results within a few hours to 2 days. But staff conducting tests need to be specially trained in molecular work and biosafety practices. Laboratories need to be equipped with appropriate biosafety infrastructure and state-of-the-art equipment. POCT potentially shortens the decision-making time for public health officials to on diagnosis, treatment, isolation, discharge and transferring of persons suspected of COVID-19.

Serological Tests

The humoral immune response following SARS-CoV-2 infection lead to production of IgM, IgA and IgG specific antibodies. These specific antibodies are directed against the SARS-CoV-2 structures including nucleocapsid protein (N) and spike (S) protein. There are still many knowledge gaps in antibody kinetics and dynamics of SARS-CoV-2 infection which is further limited by non-standardised analytical performance of commercially available immunoassays.

IgM seroconversion can be detected as early as 5 days following symptom onset, while IgG in 14 days [23]. One comprehensive study by Long et al. found that the serological conversion of IgM and IgG in COVID-19 patients can occur simultaneously (synchronous) or in asynchronous manner, either preceded by IgM or IgG. Interestingly, this study also demonstrated that serum IgA specific antibodies were detected early in the disease course and with higher positivity rate than IgM [24].Many studies have analysed the seroconversion of IgM and IgG in COVID-19 patients. Both IgM and IgG antibody levels rapidly increase after one week of symptom onset [25,26]. Positivity rate of IgM and IgG was reported to be about 85% and 78%, respectively in the first week of symptoms onset [23]. Depending on immunoassays, IgM positivity rate increased to almost 95%, while IgG were between 80-100% in the third week of symptom onset [24,27]. Although manufacturers tend to produce combination of IgM/IgG rapid tests, an evaluation study concluded that incorporating IgM to IgG measurement does not increase the detection rate of COVID-19 [28]. Median time for positive detection of IgM and IgG specific antibodies was 4-5 days following positive RT-PCR assay [29]. IgM reached its highest level in the second week and plateaued then after [30]. Positivity rate of IgM was reported to drop after 35 days and the longest was detected until 42 days following symptom onset [26,31]. IgG levels on the other hand, continued to rise in the third week and remain detected for a longer period following seroconversion [31,32]. Both IgM and IgG levels plateaued at almost one week after the initial serological detection (Figure 1). This observation may restrict the sampling window of the first sample for detection of four-fold increase in IgG in COVID-19 patients [24]. However, comprehensive longitudinal studies are required to further confirm these findings.

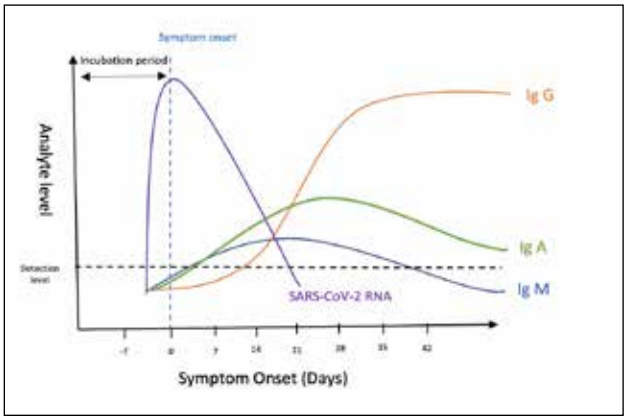

Figure 1: Estimation of viral load levels and specific antibodies during SARS-CoV-2 infection

As has been discussed earlier, real-time RT-PCR assays of respiratory samples are limited by several factors which may lead to false negative results. Serological immunoassays on the other hand, are limited by the fact that humoral immune response requires some time for specific antibodies to be synthesised and detected in the plasma. Therefore, serological testing is not suitable for screening or diagnosing recent COVID-19 infections. Several studies, however, have looked at the potential of serological assays in complementing the nucleic acid testing (NAT) in SARS-CoV-2 infection [26,33]. Specific IgM and IgG antibodies were shown to help in identifying asymptomatic patients especially among close contacts of COVID-19 patients, suspected COVID-19 cases with persistently negative NAT and those who have had past exposure to SARS-

CoV-2 [34–36]. Due to low specificity of 73%, SARS-CoV-2 IgA on the other hand is not recommended for screening purpose. Rather, it is recommended for disease monitoring of confirmed COVID-19 infections [37].

Among the immunoassay platforms that have been described and are currently available for detection of SARS-CoV-2 specific antibodies include enzyme-linked immunoassay (ELISA), chemiluminescence enzyme immunoassay (CLIA) and lateral flow immunoassay (LFIA). Generally, recombinant viral nucleocapsid and spike proteins are used as target antigens [38]. Some immunoassays use receptor-binding domain (RBD), which is part of spike protein [39]. RBD-directed antibodies are shown to have virus neutralisation properties, which is essential in measurement of protective antibody in COVID-19 vaccination study. Many SARS-CoV-2 immunoassays have been developed by different manufacturers. However, lack of standardisation and official validation question the reliability of these tests [40]. Several ELISA and lateral flow assays, however, are listed by the US Food and Drug Administration (FDA) for Emergency Use Authorisation in COVID-19 [38]. Cross-reactivity cannot completely be eliminated between SARS-CoV-2 serological assay for COVID-19 and other respiratory viruses, particularly coronaviruses. Among human-infecting coronaviruses, SARS-CoV-2 shows the highest homology with SARS-CoV. They share about 90% amino acid identity of N protein and roughly 75% amino acid identity of S protein [41]. This information indicates that better specificity of serological immunoassay can be achieved by selecting S protein or RBD as target antigens [24].

ELISA can be carried out in automation to cater high sample volume. It is less time consuming than NAT and does not require specialised operator. These advantages are particularly useful in conducting a surveillance study. Rapid detection kits on the other hand, are easy to perform and can provide rapid (within 15 minutes) results as a pointofcare testing. The reliability of rapid detection kits that employ the lateral immunoassay technique was shown to be good and comparable to other techniques [31,34,42]. Both techniques above used recombinant SARS-CoV-2 proteins, thus can be carried out widely as compared to NAT which requires laboratory with high biosafety facilities [39].

Serological data is essential for epidemiological studies as it can determine the rate of SARS-CoV-2 seroconversion in the population. It can also identify subclinical and previously undiagnosed COVID-19 infections in the community. This information is important for characterising the COVID-19 disease course, as well as for public health surveillance and future planning [43]. The WHO and CDC for the time being, only recommend the use of commercially available serological testing for surveillance and research purpose, apart from for determining recovered COVID-19 patients with high titre of convalescent plasma[44–46]. As of now, all positive serological screening have to be confirmed with NAT [47]. The role of SARS-CoV-2 specific antibodies in evaluating the disease severity and prognosis is still unclear. The specific antibody response from several studies demonstrated highly variable findings that require further evaluation on the potential use of serological testing for the purpose disease monitoring [25,30,31].

The major challenges in COVID-19 serological testing is determining the right timeto perform the immunoassays and interpreting the results. This can be achieved by understanding the antibody kinetics of COVID-19 infection over time. Determination of sensitivity and specificity of an immunoassay is important for obtaining meaningful results [39]. Use of validated serological assays is crucial especially for screening an acute infection. Serological testing is particularly useful in patients with low viral load which may be below the detection limit of RT-PCR assays. Further studies are required to ascertain whether the presence of SARS-CoV-2 specific antibodies indicate immunity and protection against re-infection. Recently, a study has demonstrated that the levels of neutralising antibodies among COVID-19 patients were highly variable and positively correlated with older age [48]. At this state, more comprehensive studies are needed to evaluate and determine the antibody types, levels and duration of protection conferred on seroconverted COVID-19 patients.

Rapid and Point of Care Tests

POCT or near patient test (NPT) are performed at or near the patient's site and detects microbial antigen or antibody extracted from clinical samples. These tests are rapid, robust, safe, simple, cost-efficient and can be performed with minimal requirements of training, biosafety and infrastructure[49–51]. Majority of POCTs for existing infectious diseases, such as HIV antibody detection utilises LFIA for detection. As LFIAs for antigen detection is dependent on the analyte concentration in a clinical sample, false negative results may be produced when the

analyte concentrations are below the assay LOD. Hence, timing of testing will have an effect on the test result. Therefore, a negative rapid test should be interpreted with caution. Emergent technologies improve accessibility, test performance and end-user adoption. Molecular technology enables nucleic acid-based approaches whereby, microfluidic devices use channels to transport small amounts of fluid by actuation forces. On-chip immunoassays reduces assay complexity and enables multiplex analysis, thus a high-thoroughput screening is achieveable[50,52,53].

With the extensive mortality and morbidity worldwide, the global community have seen the urgent need to develop POCT to screen and detect patients with COVID-19 or harbouring the SARS-CoV-2 virus. The overwhelming, unprecedentedreliance on RT-PCR testing of COVID-19 samples are a burden on central laboratories that needs to be alleviated. To date, 13 in vitro diagnostics (IVDs) detecting SARS-CoV-2 nucleic acid has been listed in the WHO Emergency Use Listing[54]. Whereby many more IVDs are being developed and still under listing assessment by the WHO, local regulation bodies such as the Food and Drug Administration (FDA) and non-profit organisations such as the Foundation for Innovative New Diagnostics (FIND). Readily available point of care platforms such as the Xpert® (Cepheid, Sunnyvale, CA) and ID NOW™ (Abbot, Scarborough, ME) have been explored for COVID-19 testing. As of 26 June 2020, the FDA US has issued Emergency Use Authorisation (EUA)S for 118 IVDs, consisting of mainly molecular based IVDs (Table 1)[55].

T**able 1:** In vitro diagnostics issued EUA by the FDA US (From 4 February to 26 June 2020)

Analyte	Technology*	Number of IVD
Gene detection	Molecular	92
IgG	CLIA	3
IgG and IgM	Lateral Flow	7
IgG	ELISA	5
Total Antibody	CLIA	5
IgM and IgG	CLIA	1
Immunoassay-IL-6	Immunoassay	1
IgG	CMIA	1
Total antibody	ECLIA	1
Total antibody	FMIA	1
Total antibody	ELISA	1

*CLIA=chemiluminescence immunoassay; ELISA=enzyme-linked immunosorbent assay; ECLIA=electrochemiluminescence immunoassay; FMIA=fluorescent microsphere immunoassay; CMIA=chemiluminescent microparticle immunoassay.

Current NPTs utilising PCR technology includes Cepheid Xpert ®, Credo Vita PCR assay, whereby Abbott ID NOW ™ utilises isothermal nucleic acid amplification techniques (LAMP). SARS-COV-2 detection by LAMP have shown similar sensitivity with the gold standard test; i.e. RT-PCR, with a detection limit of 20-fold diluted sample and uses six to eight primers to detect different regions on the targeted RNA. However, LAMP technology is still being developed and on-going assessment ensues for its clinical application. COVID-19 tests using smaller handheld devices have been developed by MesaBioTech; i.e. Accula and Microsens Dx i.e. RapiPrep©. Validation and information of potential POCTs candidates is paramount and integral in the clinical pathway[4].

Testing Strategies: Malaysia's Response to COVID-19

At the time of writing, the number of cases and deaths are dominated by the historically rich countries in the West notably the United States of America, Russia and the United Kingdom. Meanwhile, countries in the East have shown rapid, effective and innovative pandemic response to keep the incidence and mortality rate low. Screening strategies can be broadly divided into screening targeted populations at risk of infection and mass screening for all. Developed countries like South Korea, Singapore and Germany have the capability to increase testing capacity up to 1 test per 200 population enabling mass screening, isolation and control of the outbreak[56].Due to limited resources, Malaysia, an upper middle-income country with a population of 32 million people opted for targeted screening.

A joint effort between the Ministry of Health, Ministry of Higher Education and Ministry of Science, Technology and Innovations increased laboratory's diagnostic capacitiesto 36,812 RT-PCR test per day at the time of writing. Hospitals were converted to cater for dedicated COVID-19 caseswith numerous other quarantine centres in preparation for a predicted surge in COVID-19 cases. All positive COVID-19 individuals whether asymptomatic or symptomatic were isolated from the community and treated. The overall public health preparedness and response towards COVID-19 pandemic includes:i) awareness of case management among health care workers; ii) strengthening point of entry screening; iii) risk communication; iv) infection control and prevention and v) enhancing surveillance of influenza like illness (ILI) and severe acute respiratory illness (SARI)[57,58].

From 18 March 2020 onwards, the Malaysian government enforced the first phase of movement control order (MCO) and also published a federal gazette that restricts individuals from travelling to other states that have been declared as coronavirus-affected areas. In the initial phase, due to limitations in testing capacity, only symptomatic patients, individuals who had history of travel to China or contact with COVID-19 positive patients were screened for COVID-19 using nasopharyngeal and oropharyngeal swab RT-PCR. After initiation of MCO, all all international arrivals entering the country via land, air and sea routes at all border checkpoints were screened with RT-PCR and had to undergo mandatory 14 days quarantine at gazetted facilities. Prior to discharge from quarantine centres, at day 13 another round of screening was done using antibody rapid test kits. In addition to point of entry screening, eight other groups were targeted for COVID-19 screening among which included a religious event cluster, healthcare workers, senior citizens at care home and foreign migrant workers [59].

Conclusions

In order to contain this pandemic, scientists worldwide have explored established technologies and developed new in vitro diagnostics enabling rapid identification and sequencing of the SARS-CoV-2 virus. There is a need for diagnostic technologies which require minimal handling of samples and plug-and-play platforms to enable sample processing at a larger scale. The race is now to further venture in developing a POCT and multiplex assays for rapid identification, isolation, treatment, screening and surveillance.An important lesson that has been learnt from this outbreak has been that fast response in identifying index case and close contacts is as crucial as increasing testing capacity [60].

Ethical clearance

No ethical clearance was required for this review article.

Conflict of interest

The authors declare there are no conflict of interests.

Author contribution

All authors contributed to the writing and editing of the manuscript.

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