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Detecting the Coronavirus (COVID-19)

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ABSTRACT: The COVID-19 pandemic has created huge damage to society and brought panics around the world. Such panics can be ascribed to the seemingly deceptive features of the COVID-19: compared to other deadly viral outspreads, it has medium transmission and mortality rates. As a result, the severity of the causative coronavirus, SARS-CoV-2, was deeply underestimated by the society at the beginning of the COVID-19 outbreak. Based on this, in this review, we define the viruses with features similar to those of SARS-CoV-2 as the Panic Zone viruses. To contain those viruses, accurate and fast diagnosis followed by effective isolation and treatment of patients are pivotal at the early stage of virus breakouts. This is especially true when there is no cure or vaccine available for a transmissible disease, which is the case for current COVID-19 pandemic. As of January 2021, more than two hundred kits for the COVID-19 diagnosis on the market are surveyed in this review, while emerging sensing techniques for SARS-CoV-2 are also discussed. It is of critical importance to rationally use these kits for the efficient management and control of the Panic Zone viruses. Therefore, we discuss guidelines to select diagnostic kits at different outbreak stages of the Panic Zone viruses, SARS-CoV-2 in particular. While it is of utmost importance to use nucleic acid-based detection kits with low false negativity (high sensitivity) at the early stage of an outbreak, the low false positivity (high specificity) gains its importance at later stages of the outbreak. When a society is set to reopen from the lock-down stage of the COVID-19 pandemic, it becomes critical to have antibody based immunoassay kits with high specificity to identify people who can safely return to the society after their recovery of SARS-CoV-2 infections. Given that the emergence of mutant viruses at the beginning of 2021 has complicated current battle against the COVID-19, we also discussed approaches and guidelines to detect viral mutants in the middle of the second wave of the pandemic that started at the end of 2020. Finally, since a massive attack from a viral pandemic requires a massive defense from the whole society, we urge both government and private sectors to research and develop more affordable and reliable point-of-care testing (POCT) kits, which can be used massively by the general public (and therefore called as massive POCT) to contain Panic Zone viruses in future.

1. Background

Since the beginning of the 21st century, our world has been facing unprecedented crises of deadly viruses such as Zika, Ebola, SARS, and MERS. The epidemics of these viral diseases were sparked either by the evolution of pre-existing viruses or by the emergence of new viral species. These diseases have already caused colossal damage to the society. Loss of lives struck the most, but the consequences aftermath was equally dreadful: the psychological wellbeing of survivors and socio-economic fallout were rather distressing. Now, in December 2019, the world was hit by another virus known as SARS-CoV-2 (the disease associated with this virus is called COVID-19).

[Figure 1]

1.1. The Panic Zone viruses

Compared to other viruses, SARS-CoV-2 has a medium reproduction rate (R_0=2.25*) and a medium mortality rate of 5.7%*[1] (as of June 7, 2020, *subject to change). Such mediocre characteristics give a rather deceiving impression of this virus. When the virus first started in China, it did not draw immediate attention to the public due to its seemingly "benign" appearance. Indeed, compared to the Death Zone viruses which include Ebola and smallpox (see definition in Figure 1), this disease was considered merely as another type of influenza even among health professionals. However, the virus soon revealed its damaging nature. Staying untreated, the disease spread out quickly to overwhelm the health systems in a society. This eventually caused panics in the general public. People rushed to see doctors even if they developed very mild or even unrelated symptoms, which overran hospitals. This is because in modern society, the production system of healthcare supplies is profit driven[2]. Decisions regarding the management of disease can no longer be made based solely on scientific grounds. Unless a disease poses a specific risk to a wide population, its mere presence in a localized area or population may not be significant from a business perspective. As a result, necessary resources such as PPE (Personal Protective Equipment) are in short supply to fight pandemic diseases promptly. Due to these reasons, the diseases in the Panic Zone (see Figure 1 for definition) often wreck huge collateral damages due to its paralyzing role for the whole society.

In the Panic Zone, SARS was most recently contained by means of massive syndromic surveillance, prompt isolation of patients, and strict quarantine of all contacts. By interrupting all human-to-human transmissions, SARS was effectively eradicated in 2003[3]. Although there are striking similarities between SARS and COVID-19, the differences in the virus characteristics will ultimately determine whether the same measures for SARS will also be successful for current COVID-19 outbreak. COVID-19 differs from the SARS in terms of infectious period, transmissibility, clinical severity, and extent of the community spread[3]. Although COVID-19 has lower transmissibility than

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SARS[4], many more COVID-19 patients have mild symptoms that contribute to the rapid spread of the virus as these patients are often missed and not isolated.

1.2. The early detections

It is generally true that for a rapidly transmitting disease with no cure or vaccine available, the most effective way to curb its spread is the early detection to isolate patients[5,6]. The first step to achieve this is to identify those patients using detection kits. Never before is a virus detection system so critical to contain a viral outbreak as dangerous as COVID-19. As shown in Figure 2, for the five countries with similar age distribution and hospital resources, the more extensive the early tests on the COVID-19, the lower the overall mortality rates in a country. Indeed, Korea and Germany conducted a substantial number of the tests right at the beginning of the COVID-19 outbreak. Correspondingly, their death rates are among the lowest so far (Figure 2, inset). This confirmed the importance of the early testing to curb the spreading of the COVID-19.

[Figure 2]

In this review, we first describe the COVID-19 outbreak briefly. Given the importance of the diagnosis for this deadly pandemic disease, we then survey the detection kits used for the COVID-19. After summarizing the challenges facing current commercial kits, we discuss emerging techniques to address these issues. Next, we propose and discuss guidelines to use various kits during different stages of the COVID-19 outbreak, including current stage when SARS-CoV-2 mutants became prevalent. Finally, we wrap up by proposing more extensive research and development of affordable point-of-care testing (POCT) kits that can be used massively (massive POCT) to battle these viral pandemics in the future.

2. The COVID-19 outbreak

2.1. COVID-19 Timeline

In December 2019, a cluster of pneumonia cases were reported in Wuhan, China[7]. The causative virus of that disease was determined as SARS-CoV-2 (later this disease was called as COVID-19, Corona Virus Disease 2019, by the WHO) since the virus shared ~80% genome from the SARS-CoronaVirus[8]. On January 11, 2020, the first death caused by this virus was reported in China. This disease was highly contagious and therefore was declared by the WHO as a Public Health Emergency of International Concern (PHEIC) within a month after the first case. On March 11, WHO declared COVID-19 a pandemic disease as it started to spread across the globe.

2.2. Clinical characteristics of COVID-19

Based on current epidemiological researches, the clinical characteristics for COVID-19 appeared in 1-14 days after the infection and most patients developed symptoms within 3-7 days[9]. The common symptoms include fever, coughing, and body weakness. A few patients developed nasal congestion, running nose, pharyngalgia, myodynia, and diarrhea. In severe cases, by the end of the first week, the disease can develop into dyspnea and/or hypoxia. In deadly cases, the disease can quickly progress to acute respiratory distress syndrome, septic shock, coagulation disorders, and multiple organ failure[9]. It is noteworthy that patients with high viral loads may have low or insignificant fever during the infection. Some children and neonates did not have typical symptoms, but they presented with gastrointestinal symptoms such as vomiting and diarrhea or presented with depression or shortness of breath[10]. The elderly and patients with chronic underlying diseases had poor prognosis[11].

2.3. Epidemiology of COVID-19

People are generally susceptible to the SARS-CoV-2 infection at all ages. The infection is transmitted by droplet (direct inhalation of droplets from the sneeze, cough, or talking of an infected person) or contact (contacting the virus deposited on the object surface, which then enters the body via the mouth, nose, eyes, or other mucous membranes[12]). Study showed a higher viral load in the nasal cavity than the throat, suggesting the nasal sampling is a more effective approach to detect the virus. There was no difference in the viral load between symptomatic and asymptomatic patients[13], the latter of which can also transmit the disease[14]. Guan et al. reported that some patients were tested positive for SARS-CoV-2 in stool and urine samples also[9].

3. Diagnosis of the COVID-19

As discussed in the Background, in the absence of effective therapeutic drugs or vaccines for COVID-19, it is essential to detect the disease at its early stage and immediately isolate infected patients. Currently, there are three methods in clinical practice to diagnose COVID-19, which are summarized below.

3.1. Chest CT Imaging

Studies showed that chest CT images contained characteristic features for COVID-19 patients. The hallmarks of these CT images include ground glass opacities, crazy-paving pattern, consolidative opacities, septal thickening, and the reverse-halo sign[15–18]. These features demonstrate a highly organized pattern of pneumonia[16]. Unlike these features, nodules, cystic changes, bronchiectasis, pleural diffusion, and lymphadenopathy are less common[18].

Despite such features, the Centers for Disease Control (CDC) in the US does not currently recommend CT to diagnose COVID-19. Laboratory testing of the virus remains the reference standard, even if the CT findings are suggestive of SARS-CoV-2 infections[19]. This is because features of the chest imaging from COVID-19 patients may overlap with other infections caused by influenza, H1N1, or SARS-CoV[20,21].

However, studies on the sensitivity of CT imaging over RT-PCR (Reverse Transcription - Polymerase Chain Reaction, which is considered as the reference standard for laboratory testing of SARS-CoV-2, see section 3.2 below) showed that CT imaging can be more sensitive and rather reliable in detecting SARS-CoV-2 infections during certain stage of the COVID-19. Fang et al. studied 51 patients with COVID-19 symptoms based on their clinical manifestations and epidemiological histories[22]. They found that the chest CT scan was more sensitive (98%) than the RT-PCR method (71%). This study was limited by the number of subjects involved. However, another study involving more than 1000 patients reached similar conclusions[23]. Among 1014 patients, 59% were RT-PCR positive, from which 97% showed positive CT features. In addition, 75% of RT-PCR negative patients showed positive CT features. To further validate this, Ai et al. studied multiple RT-PCR testing and serial CT imaging in a selected group. They found 60 - 93% people who were RT-PCR negative showed initial positive CT images consistent with SARS-CoV-2 infections. From the patients in the recovery stage, 42% showed improvement in CT features before their RT-PCR results turned negative.

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According to these diagnostic studies, RT-PCR assays were not as sensitive and reliable as CT images in certain stages of the COVID-19. The false negative results from RT-PCR assays can be detrimental to the control of the COVID-19, especially at the beginning of the outbreak. The caveat for the CT scans is that at an early stage of infection, the lungs of a patient may not develop damaging features that can be picked up by CT scans, increasing its false negative rate. In addition, the COVID-19 CT features share similarities with other viral pneumonia, resulting in false positive detections. Nevertheless, given the rapid spreading of the COVID-19, the priority is to identify any suspicious case for patient isolation and proper treatment. In the context of emergency disease control, some false-positive cases (i.e., compromised specificity) may be acceptable. It is the false negative cases, due to the poor sensitivity of testing methods, that present a threat to public health at the beginning of an outbreak. In some cases, chest CT imaging showed positive SARS-CoV-2 infection while RT-PCR testing was negative[22]. These findings suggest that a combination of clinical symptoms, epidemiologic history, and CT imaging of a patient may be instrumental to identify SARS-CoV-2 infections at the time when chemical detection kits are in short supply.

3.2. Nucleic acid-based methods

After identification of the SARS-CoV-2 as the causative virus for this pandemic, the SARS-CoV-2 genome was quickly sequenced[24], from which unique sequences have been identified for COVID-19 diagnosis. Reverse transcriptase-polymerase chain reaction (RT-PCR) is a nucleic acid amplification assay that has long been used routinely for the detection of RNA viruses in clinical settings[25]. In RT-PCR, reverse transcriptase is first used to convert RNA to its complementary DNA, which is amplified by PCR (polymerase chain reaction). There are variants of RT-PCR methods that share the same mechanism while differing in the detection strategy. For example, real time RT-PCR reads fluorescent signals during PCR amplification[26] to quantify the target, whereas nested RT-PCR uses two sets of primers to avoid non-specific PCR amplifications[27].

The SARS-CoV-2 genes targeted for detection so far include the RdRP gene (for RNA dependent RNA Polymerase), Nucleocapsid (N) gene, Envelope (E) gene, Spike protein (S gene), and ORF1ab gene (for the Open Reading Frame 1ab region). Chu et al. used two different one-step real-time RT-PCR approaches to detect ORF1ab and N genes of the viral genome[28]. This assay showed a high dynamic range of 0.0002-20 TCID50 (50% Tissue Culture Infective Dose) per reaction and the detection limit below 10 RNA copies per reaction volume of 4 µL. Later, WHO developed a technical guidance including the protocols from different countries to aid COVID-19 diagnosis[29]. According to this compilation, in the US, CDC developed a real time RT-PCR diagnostic kit with detection limits as low as 4-10 RNA copies per µl. Scientists from Germany used the E gene for the first-line screening and the RdRP gene for confirmatory testing[29]. This method further increased sensitivity to detect as low as 5.6 RNA copies per reaction (25 µL) for the E gene and 3.8 RNA copies per reaction for the RdRP gene. In Hongkong, the N gene was used as the first-line screening while the ORF1b as the confirmatory testing[29]. In France, two RdRP genes were used for initial screening followed by the confirmatory E gene testing[29]. In Japan, nested RT-PCR was used,[29] which significantly reduced non-specific target amplification, leading to decreased false-positive results (i.e. increased specificity). In general, the sensitivity of these assays ranges from 3.8 to 10 RNA copies per reaction of 5 µl, with high specificities.

In the public health emergency, highly sensitive methods are desirable. Although studies have shown that RT-PCR may be less sensitive than CT imaging at certain stages of the COVID-19, its specificity makes it superior to other methods to detect SARS-CoV-2. It is of critical importance to rationally choose specific diagnostic methods to battle viral outbreaks. Any negligence or compromise in the diagnosis may lead to devastating consequences. Wang et al. suggested combining RT-PCR with other methods as well as epidemiological history of patients to diagnose SARS-CoV-2 infection more credibly[30]. Indeed, the Chinese authority has adopted this approach to diagnose COVID-19 in Wuhan by combining RT-PCR with CT scans[23]. Studies also showed that the sensitivity of RT-PCR varied with the specimen types. To et al. revealed that saliva samples were more promising to be used in RT-PCR[31] while Yam et al. concluded that testing more than one specimen could significantly maximize the sensitivity of the RT-PCR testing[32]. These findings suggested it is rather important to apply nucleic acid-based kits with optimized conditions to maximize their diagnosis potency. In particular, the finding of effective SARS-CoV-2 detection in the non-invasive saliva sampling[33] has provided a convenient way to develop affordable point-of-care testing kits that can be massively used by the general public (see Sections 4 and 5 below).

Table 1 lists the nucleic acid-based kits for the diagnosis of COVID-19. The sensitivity of those kits ranges from 100-1000 copies/mL.

3.3. Immunoassays

Immunoassay is another established diagnostic method (Table 2). This method detects viral protein antigens (the antigen test) or blood antibodies (the antibody test) in patients who have been exposed to the SARS-CoV-2. These immunoassays are important in detecting prior infections.

Antigen tests aim to detect viral proteins. Studies have been reported that the SARS-CoV-2 virus expresses four major structural proteins, spike (S) protein, nucleocapsid (N) protein, envelope (E) protein, and membrane (M) protein. The S protein recognizes the host cell receptor ACE2, which then promotes cellular entry of the virus[34]. Thus, S protein determines the infectivity of the SARS-CoV-2. Comparing to the SARS, the binding affinity of the S protein to the ACE2 was 10-20 times stronger. The highly conserved N protein is the most abundant protein in this virus. During the assembly of virions, N protein binds to the viral RNA and encapsidates it to form a helical nucleocapsid. It is also involved in the genome replication and regulation of cell signaling pathways[35]. M protein also plays central roles in assembly via interacting and binding with various other viral proteins[36]. The E protein is an integral membrane protein involved in various processes including assembly and pathogenesis[36]. As an integral protein, most of this protein is not exposed to aqueous solution. In addition, this protein is less abundant than S and N proteins, poorly immunogenic for humoral responses, and has small molecular size, the latter two of which are the same for the M protein[37]. Therefore, for better sensitivity of antigen tests, the S and N proteins are widely exploited as targets. From the SARS-CoV-2 topology[38–40], S protein sticks furthermost into the solution compared to other proteins, making this protein the best target for antigen test. However, S protein is prone to undergo mutations. Recent discovery of a highly infectious strain has mutations in this protein[41],

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raising concerns of false negativity in the antigen test targeting the S protein. In fact, amid the second wave of COVID-19 pandemic started at the end of 2020, the rapid spread of this type of mutant virus has caused alarms across the world (see section 6.2 for discussion). If binding antibodies currently used in the antigen tests only recognize mutable sites of a target such as the S protein, it is possible that false negative result will be obtained against particular SARS-CoV-2 variants. From this perspective, polyclonal antibodies that target different sites of S protein are expected to be more reliable to detect mutant virus.

In antibody tests, antibodies against SARS-CoV-2 are detected in patient blood. In the SARS-CoV-2 infection, studies have shown that the seroconversion in the patient generally starts after a week of the first symptom[42]. In a study of post symptomatic patients, Amanat et al. detected high IgA and IgM immune responses[43]. Using recombinant viral proteins, this immunoassay could detect antibodies as early as 3 days after the development of the first symptom. Liu et al. reported that the accuracy of the ELISA for IgG and IgM antibodies was more than 80%[44]. The efficacy of the immunoassay also depends on the specificity of the antigens used to capture the antibodies from the patients. Between the spike (S) proteins and nucleocapsid (N) proteins, the sensitivity of the S proteins is higher for the antibody capture. Among various spike proteins, the S1 protein has shown more capabilities to bind to SARS-CoV-2 antibodies[45]. In a comparative study, both ELISA and colloidal gold immunochromatographic kits showed equal sensitivity with 100% specificity for the SARS-CoV-2 detection[46].

Many immunoassay kits are already on market for emergency detections of COVID-19 specific antibodies (see Table 2). However, the major problem of this method is that it only works for infected patients who must have an immune response to the SARS-CoV-2. At this stage, some patients may already be critically ill. Other drawbacks of immunoassay include changes in viral load over the course of infection[47], potential cross reactivity (less specific)[48], and low sensitivity with respect to nucleic-acid based methods. Nevertheless, immunoassays are faster[49] and cheaper than the RT-PCR methods. Antibody tests can be used for rapid screening of previous SARS-CoV-2 infections. This is particularly useful in the reopening stage of the society at which people recovered from previous COVID-19 infections and therefore, immune to the virus, can safely re-engage to the society. The method also has a unique advantage of identifying individuals who have strong immune responses against the virus and therefore, can serve as potential donors for therapeutic and research purposes.

4. Ideal characteristics of diagnostic methods

Diagnostic testing has become indispensable for diagnosis, prognoses, and monitoring the progress of different diseases. Efficient diagnostic testing is an important intervention for pandemic management and control. WHO has developed the **ASSURED** criteria as a benchmark to decide if a test efficiently addresses the needs for disease control: **A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free and **D**eliverable to end-users[50]. It is ideal to have all the criteria fulfilled in a single test. In practice, however, testing methods can rarely fit all the ASSURED criteria. In pandemics for example, rapid and sensitive methods are dearly needed at the beginning of an outbreak. But many available kits require qualified laboratories and personnel for testing. In such a case, accommodation of the ASSURED principles must be taken to facilitate the testing.

In a pandemic, it is always important to understand the nature of the pathogen before developing efficient diagnostic tests. Translating the tests into the point-of-care (POC)[51] mode can help decision-making and improve the efficiency of the treatment. POC provides rapid and actionable information for patient management and care at the time when it is most needed. Many affordable POC antibody and antigen test kits such as lateral flow immunoassays[52] (see Table 2) are also appropriate for resource-limited settings in middle- or low-income countries where laboratory infrastructure is weak. One example for affordable POC testing is the SARS-CoV-2 Antigen Test Kit[53], which only requires nasopharyngeal swabs instead of more intrusive blood sampling with results obtained within 10 minutes. Due to the requirements of easy usage and cheap price, they often use colloidal gold-based immunoassay mechanisms, where the monoclonal antibodies are functionalized on gold colloids to improving the sensitivity. Such POC testing kits perhaps represent the best solution to fight fast transmitting pandemics.

5. Emerging techniques to detect SARS-CoV-2

Given a variety of problems associated with current clinical diagnosis for the SARS-Cov-2 (Section 3), here, we discuss some promising techniques that may address these issues.

5.1 Isothermal amplification for nucleic acid targets

Although RT-PCR is a widely used method in the confirmatory screening of COVID-19 infections (see Section 3.2), it is time consuming and requires sophisticated laboratory facility and trained personal to operate[54]. To simplify the testing procedures, isothermal nucleic acid amplifications have been developed. These methods do not require any thermal cycler to perform the amplification and therefore, can be carried out in a simple water bath at a constant temperature of 40-65 ^{o}C[55]. One promising isothermal nucleic acid amplification approach is Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP). In this method, the RNA genome of SARS-CoV-2 is first reverse-transcribed to cDNA, which is then amplified using four to six target-specific primers. Prior to the LAMP amplification, a dumbbell shaped single-stranded DNA (ssDNA) is formed through the annealing and the strand displacing cycle on both ends of the target sequence with the help of the primers and a strand-displacing polymerase. The looped ssDNA on each end then serves as a seed for the LAMP amplification cycle[56–59]. As a result, the target sequence is amplified exponentially, which is detected by turbidimetry[60] or fluorescence[61]/colorimetry[56].

As an example, the RNA extraction and LAMP amplification have been performed in the same tube[59,62]. This method has the LOD ranging from 80 to 500 SARS-Cov-2 RNA copies per milliliter, which is comparable to the RT-PCR assay. To improve the LOD, El-Tholoth et al. developed a two-stage closed tube test (named Penn-RAMP) by combining LAMP with Recombinase Polymerase Amplification[63], which is another widely used isothermal method for nucleic acid amplifications. In the Penn-RAMP, each amplification was performed at a separate compartment in a single tube followed by mixing. The method demonstrated 10 times higher sensitivity than LAMP or RT-PCR alone. In other developments, Zhang group[64] and Chiu group[65] integrated the LAMP with the CRISPR-based SHERLOCK (see Table 1)[66] and CRISPR-Cas12 based

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methods, respectively, to detect the SARS-Cov-2 RNA with a detection limit as low as 10 copies/µl on a point-of-care testing (POCT) format. Some commercial COVID-19 diagnostic kits based on isothermal RT-LAMP assays are already on the market (see Table 1). Abbott ID Now™ COVID-19 is such an example. This method only requires 5 minutes to give positive results. Recently however, issues on the false negativity have been raised for the Abbott ID Now™ because of its relatively high LOD [67]. This may be attributed to the compromised performance of the RdRP target[68,69] used in this assay, which is found to be mutating and evolving[70].

Rolling circle amplification (RCA)[71] is another isothermal amplification method that gives sensitive detection of nucleic acids. In this method, a segment of the target genome is circularized and amplified by a highly processive strand-displacing DNA polymerase. Wang et al. used this method to develop a highly sensitive and efficient assay for SARS-CoV[72]. Compared to the LAMP assay, the RCA method is simpler since it requires fewer steps, and it can be performed at room temperature. The method can offer sensitivity comparable to RT-PCR[73] since it amplifies the target sequence by at least ~10,000 folds. In addition, it presents high specificity as the RCA is initiated only after the formation of a circular template upon which a specific primer is hybridized[73]. Therefore, RCA reduces false-positive results often encountered in PCR-based assays. A major difficulty in this method is that it requires a circular template whose preparation is dependent on the length of a linear template and the ligation efficiency of the circularization. Inappropriate design of complementary sequences therefore results in the failure of amplifications.

5.2 Lateral flow-based detection of nucleic acids and proteins

The nucleic acid-based isothermal amplifications discussed above partially overcome the limitations of conventional RT-PCR assays as they do not require sophisticated laboratory facilities while their turnaround time is short. However, these methods still require trained staff to operate various sample collection and processing steps. To address these problems, paper-based lateral flow assays (LFAs) have gained interest because of their low cost, easy manufacturing, and full compatibility with POCT, which allows them to be conveniently performed by anyone at home.

In LFAs, both nucleic acid detection methods and immunoassays can be utilized. The device is often made of filter papers with immobilized capture probes. Upon binding with nucleic acid targets, the probes give a visible signal[74–76]. Such methods still require initial nucleic acid extraction and amplification steps, the latter of which can be accomplished by the PCR or isothermal amplifications as discussed above. On the POCT platform, all those steps are integrated in a single device. Reboud et. al.[76] developed a paper-plastic lateral flow method to detect nucleic acids of malaria. They used a foldable paper in which extraction of malaria genome and LAMP amplification of target sequences were performed at separate locations. The LAMP amplified DNA was carried by capillary flow to the detection zone, giving a visible color change[76]. Similarly, Byers et al developed a 2D paper network to perform immunoassay for the detection of nucleic acids of SARS-CoV-2 with the POCT format[77].

Although nucleic acid-based lateral flow assays are sensitive, lateral flow immunoassays have gained interest in the massive surveillance of COVID-19 pandemic because of their simplicity and cheap cost. Currently, both antigen (N and S proteins) and antibody (IgM/IgG) based rapid test kits are available for qualitative antibody test of COVID-19. Many such commercial devices have already been developed (See Table 2). One problem associated with the immunoassay based lateral flow assay is the weak signal, which results in reduced sensitivity[74]. Various signal enhancement strategies therefore have been proposed. A promising signal amplification strategy in lateral flow assays is the use of colloidal gold nanoparticles conjugated with the probes. Upon binding with the target, the gold nanoparticles linked to the capture probe aggregate to change the color, enhancing the signal[78]. Other signal amplification strategies include solvent evaporation for analyte preconcentrations, nanoparticle catalyzed nanoparticle labeled assays, and ion concentration polarization methods[79].

Due to the low-cost requirement of the POCT, detection in the LFA is usually achieved by visual inspection. To improve detection sensitivity, cameras in smartphones have been used[80]. These cameras are sensitive to subtle color changes and hence provide more effective color detection than traditional RGB sensors or the naked eye[81]. For improved read-out of the results and data processing, machine learning algorithm could also be used[82]. Smartphones can also be coupled with external adapters to integrate external biosensor platforms for more versatile POC testing[83].

5.3 Other emerging methods

As discussed in section 4, diagnostic tests developed so far rarely meet all the ASSURED criteria. The most important features for the SARS-CoV-2 detection are sensitivity, specificity, and efficiency (throughput and cost-effectiveness). In addition to the approaches discussed in the sections 5.1 and 5.2, other emerging methods have been developed to improve these features. To improve the sensitivity, methods with single-molecule detection capability can be used[84–86]. As an example, the single molecule enzyme linked immunosorbent assay (ELISA) has been developed to offer detection limit of sub-femtomolar protein concentrations[87]. In this method, each microscopic bead decorated with specific antibodies is loaded into individual femtoliter wells. Sensing was accomplished by the ELISA on each bead, whereas the excellent concentration detection limit was achieved by a large array of such beads. To be applied in clinical setting, however, this method requires special equipment, increasing its cost.

To increase the specificity, Proximity Ligation Assay (PLA) has been developed. The method utilizes two or more DNA-tagged aptamers or antibodies for bindings of multiple targets[88]. The DNA tags on the probes are amplified only when the two different targets are in close proximity. The multiple targets ensure the specificity of the target detection. However, this method requires intact SARS-CoV-2 virus particles from which two different targets are present for positive detections. This demands stringent sample processing steps.

To increase the throughput, fast sequencing such as next generation sequencing[89] and DNA microarray[90] can be used. In the case of COVID-19, evidence have suggested that the SARS-CoV-2 is rapidly evolving while infecting people. Therefore, it is critical to rapidly identify the genome of the causative agent[91]. The DNA microarray has been used in high-throughput identification of mutations in SARS-CoV-2[92]. However, for these methods, the time limiting step becomes the sample collection, which must be performed one-at-a-time. In addition, these methods involve rather advanced equipment with high

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cost, therefore, they may not be appropriate for the economic and rapid screening in the COVID-19 pandemic.

6. Rationales in choosing diagnostic methods in the COVID-19 outbreak

6.1. Initial breakout stage

As stated in the introduction, early diagnosis becomes one of the most important approaches to curb a viral outbreak such as COVID-19, which does not have a cure or vaccine. As shown in Figure 3, intervention such as identification of patients for isolation at the early stage before the inflection point of the viral spreading will significantly slow down the transmission of the virus. It will not only delay the time at which the peak occurs, but also reduces the magnitude of the peak population. While decreased peak magnitude directly reduces the burden on hospitals, the delay of the peak occurrence gives more time for the public to prepare well for the peak-time challenges. Both are expected to decrease the mortality rate. Such an early intervention heavily relies on the quality and quantity of the detection kits for specific viruses. Since the start of the COVID-19 outbreak, many diagnostic kits have been developed in different countries (see Tables 1-2). With the increase in the number of diagnostic tests, it is difficult for policymakers, laboratories, and other end-users to make rational decisions on the selection and use of these tests. As a result, tests have been used unnecessarily and incorrectly, with results misinterpreted. Here, based on the epidemiology of the COVID-19 and the available diagnostic kits on the market, we suggest some guidelines to rationally select kits for efficient disease control and suppression. In particular, we will discuss the relative importance of sensitivity and specificity[93,94] of different assays in the fight against the COVID-19 pandemic.

[Figure 3]

Among all current methods, nucleic acid-based kits are considered the most reliable because of their excellent sensitivity and specificity. This is not surprising since these methods target unique sequences in the viral genome for diagnosis. Due to these advantages, it becomes a detection of choice at the beginning of a viral outbreak (Figure 4). At this stage, it is critical to identify and isolate all possible patients before the virus enters an exponential growth stage (around the inflection point, see Figure 3). Therefore, it is important to reduce the false negative results of the diagnosis. To achieve this, high sensitivity is a necessity. The PCR amplification used in various RT-PCR kits can detect as low as 100 copies/mL reaction (see Table 1), which is equivalent to 0.167 attoMolar (for a reaction volume of 100 microliters). It is noteworthy that high sensitivity is often accompanied with increased false positive results[95,96]. But at the beginning of a viral outbreak, some false positive level may be tolerated. Since there are not so many infected patients at the initial stage of the outbreak, the chance of cross contamination from COVID-19 patients to these false positive cases is small, even if they are isolated together (but well protected by PPE) in spacious locations such as convention centers. When the viral outbreak becomes stronger, false positive cases should be reduced (i.e., specificity increased) as much as possible due to the increasing cross contamination concerns.

[Figure 4]

Due to the extensive amplifications, isothermal amplification-based methods[97] (see Table 1) usually have superior sensitivities albeit with increased false positive levels[95,96] (see section 5.1). Therefore, at the beginning of an outbreak, isothermal amplification may be used first. However, this method usually involves many testing steps, therefore, it is more complex to run. Due to the same reason, its development and approval also take time, which makes the technique slow to be adopted at the beginning of an outbreak. With easy performance and fast approval, PCR-based kits still remain the gold standard at the beginning of a viral outbreak.

Another means to reduce the false negativity in nucleic acid-based testing is to perform CT scans. As discussed in Section 3.1, it can be more sensitive to diagnose COVID-19 using CT scans at certain stages of the disease. The caveat for the CT scan is its relatively low specificity (i.e., high false positive results), which may be tolerated at the initial stage of an outbreak. However, positive CT scans only diagnose patients at the later stage of their SARS-CoV-2 infections, which limits its use for early-stage screening. The method is still valuable to quickly screen serious cases from mild ones. Due to limited testing kits and over-burdened clinical resources, many patients with mild symptoms have been self-isolated first. When their conditions deteriorate, it becomes important to streamline life-threatening cases as soon as these patients are sent to the hospital. Due to the fast performance and interpretation of CT scans within tens of minutes as demonstrated in China hospitals for example, these patients can be quickly identified, followed by appropriate treatment to save lives.

Antibody based immunoassays work well only after the human body develops antibodies against the viruses. Therefore, these kits are not appropriate to detect infection cases at the early stage of an infection at which patients may be asymptomatic. Given that asymptomatic patients also transmit COVID-19[14], it is not recommended to use immunoassays at the beginning of the pandemic. In the current COVID-19 breakout, we have often seen that during the exponential increase stage of the disease (around the inflection point, see Figure 3), there have been insufficient number of nucleic acid-based kits to test all suspicious cases. Current strategy to solve this issue is rather passive. These precious testing kits are reserved only for more serious cases. For the patients with light symptoms, they were sent home for self-isolation. The immunoassay can be used to test those patients after their symptoms lasted about 3 days when IgM can be detected in blood[98]. Since these immunoassays are cheaper, faster, and easier to perform[52] with respect to nucleic acid based methods, they can be quickly and massively conducted by staff at drive-thru stations or by patients themselves. This is particularly important during the society reopening stage of the COVID-19 pandemic in which the recurrence of the disease must be avoided while the lifestyle is set to be normal again (Figure 4).

6.2. Recovery from the pandemic

In this stage where the society is set to reopen, it is important to ensure that there is no recurrence of the COVID-19 breakout. To this end, one of the most important approaches is to identify people who have been previously infected with the COVID-19, and therefore immune to the SARS-Cov-2 virus. Since these people are clear of viral load, only antibody-based immunoassay detection can be used for this purpose. It is a fatal mistake for the whole society if false positive cases are high in such screening. In such cases, people who have not been exposed to the virus and therefore, vulnerable to the COVID-19, are wrongly identified as immune to the disease. This misidentification will expose them to the SARS-Cov-2 infection, which

increases the chance for the recurrence of the COVID-19 in a recovering society.

During current pandemic recovery stage in which vaccination becomes available, one of the most critical challenges to fight COVID-19 is the rapid evolution of the SARS-CoV-2. Recently, mutants of the SARS-CoV-2 are identified in many countries[99]. Such strains have altered genomic sequences which may make it difficult for existing nucleic acid-based methods especially when the mutation occurs in the complementary sequence of primer binding sites, preventing a primer from hybridization to initiate the polymerization. When mutated, viral proteins (antigens) may have altered structures which cannot be recognized by current antibodies. All these lead to false negative results.

Genomic sequencing is the most reliable tool to identify mutant strains once suspicious infections are confirmed in laboratory. When a particular type of mutation is identified, biosensing techniques could be developed to quickly detect that specific mutant virus[100]. For example, a specific aptamer, an antibody, or a nanobody could be developed to target a specific mutant protein. To target nucleic acids, a set of primers can be designed and optimized to specially identify a mutant genotype by binding to the mutating sites for downstream amplifications. Other rapid mutant detections include programmed software package which takes raw genome sequence as an input and gives mutation loci as an output after comparison with wild-type genomes in the database[101].

6.3. Preparation for future pandemics

In the future, affordable POC testing (POCT) kits as discussed in section 4 may present a viable direction to address the bottleneck diagnosis problem caused by shortage of testing kits at the beginning of any viral outbreak. These kits can be performed at home for self-isolated people with mild symptoms. If they are tested positive by the POCT kits, their conditions will be closely monitored for further medical treatments or other interventions. The inherent properties of these POCT kits (cheap, fast, and easy-to-use) afford their massive usage by the general public to fight with future pandemics. We therefore name such an approach a massive POCT strategy. It becomes critically important that such massive POCT can be developed rapidly to fight future viral outbreaks.

7. Conclusions and Perspectives

In summary, like other viruses in the Panic Zone, the SARS-CoV-2 has caused unexpected damage to society. During the outbreak of the COVID-19, most studies have focused on the potential causes and epidemiology of the virus while the information on the epidemic prevention is obscure. From the data we have collected so far, it is imperative to carry out the diagnosis to isolate and treat patients at the early breakout stage of the viruses in the Panic Zone. This is especially important for the virus without a cure or vaccine. The burden of accurate and rapid diagnosis falls on the detection kits used for the SARS-CoV-2 detection, which include nucleic acid-based methods and immunoassays for both antigen and antibody tests. Given the epidemiology of the COVID-19 and the features of available detection kits, it is crucial to reduce false negative results (i.e., increased sensitivity) at the expense of some false positive detections (i.e., reduced specificity) during the early stage of the outbreak. It becomes important to reduce the false positivity in later stages of the outbreak, especially when the society is poised to reopen from the lock-down stage. Although nucleic acid-based detection kits, RT-PCR in particular, offer best solutions so far to these requirements because of their high sensitivity and specificity, immunoassays can well supplement the detection armory due to their cheaper price, simpler operation, and faster detection time. The use of blood antibody targeted immunoassays is especially useful at the later stages of the virus outbreak which include the second wave of the COVID-19 pandemic occurred since late 2020, when people who have been recovered from the COVID-19 are identified to facilitate their reengagement to the society. When a virus is mutated, caution should be given for false negative results especially for antigen tests. We believe a massive attack from a Panic Zone viral outbreak requires a massive defense from the whole society. The best approach to deal with this massive attack is the development of cheap, fast, and easy-to-use point-of-care testing (POCT) kits that can be used in a massive fashion by the general public. In the future, intensive research and development on the so-called massive POCT kits for Panic Zone viruses therefore should be encouraged both by the government and private sectors.

NOTE FOR THIS PREPRINT

This preprint is an updated review of the SARS-CoV-2 detection described in the manuscript published in ACS Sensors [102]. In current version, we updated FDA approved SARS-CoV-2 detections kits as of 01/2021. We also added a section to discuss the detection of the SARS-CoV-2 mutants that emerged as major strains at the end of 2020.

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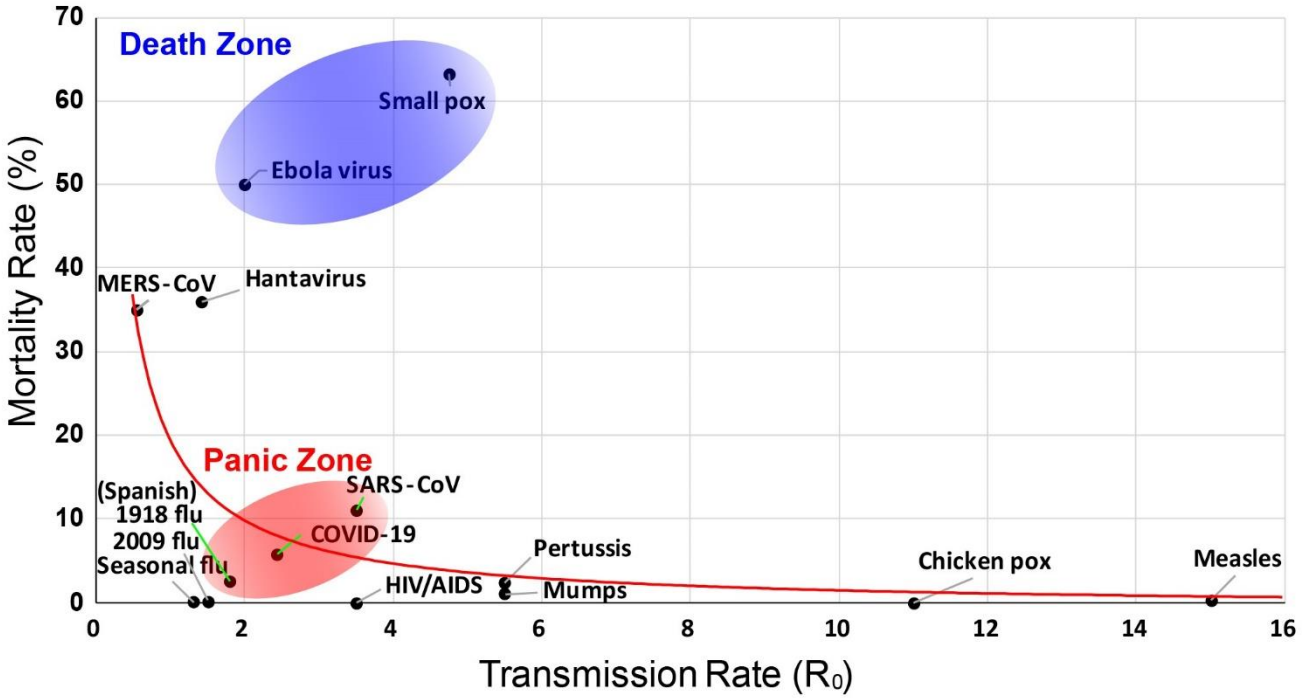

FIGURE 1. Viruses with high transmission rates (R_0) are less fatal. R_0 is the reproduction rate of a virus, which measures its transmissibility[77]. Solid curve represents an inverse fitting between the mortality rate and the R_0, which has been proposed as the trade-off principle between the virulence and transmissibility of virus[104]. The inverse function fits well except for the two viruses in the Death Zone (blue), which is defined to have a rather high mortality rate. The Panic Zone contains viruses with medium levels of transmission and mortality rates. The data used here are taken from references[105–113].

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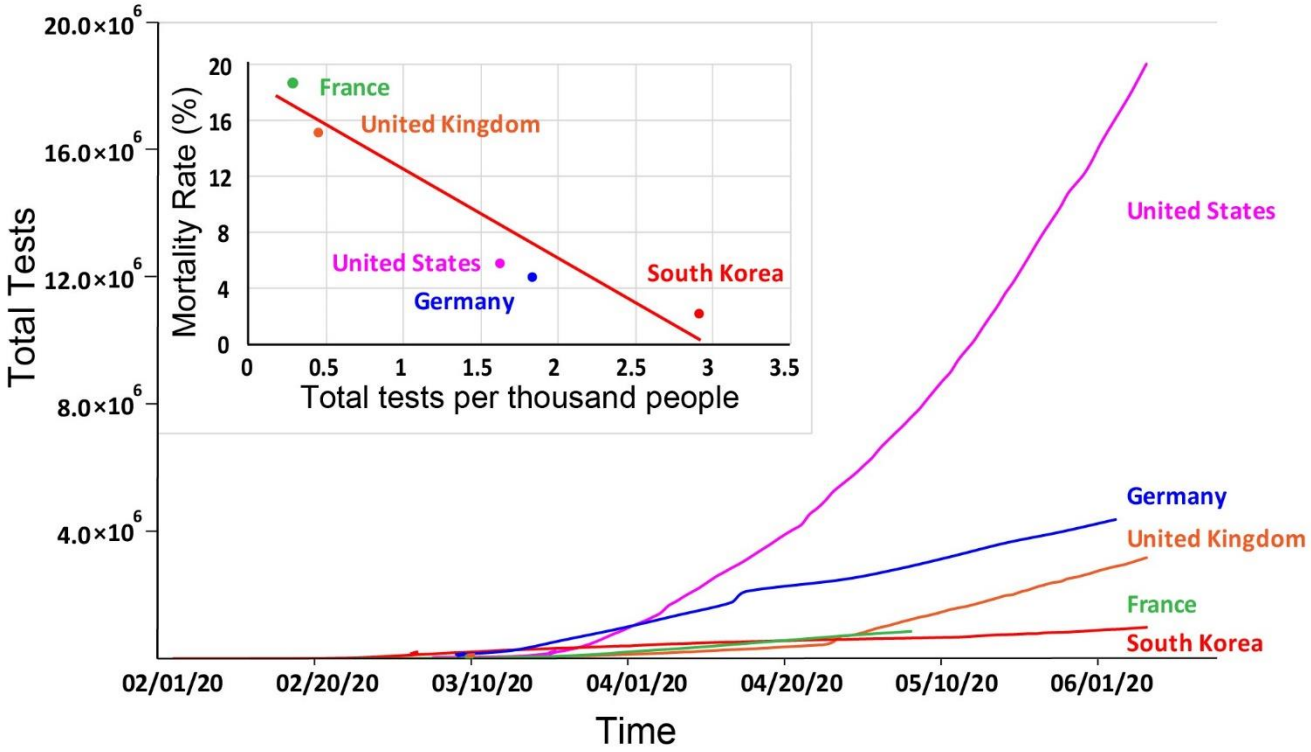

FIGURE 2. Critical importance of the early detection in the COVID-19 outbreak. COVID-19 daily tests are shown for 5 countries with similar medical resources and age distributions. Inset shows the mortality rates (percentage of the death cases among all confirmed COVID-19 cases) as of 06/07/2020 vs the number of the early detections per thousand population performed during 03/04/2020 - 03/26/2020. The early detection data for each country[114] are taken from different periods (marked by stretches) to reflect the timing of the outbreak in Asia, Europe, and North America (~2 weeks apart). The inset data are linearly fit (r=-0.94), which indicates a negative correlation between the early detection and the mortality rate.

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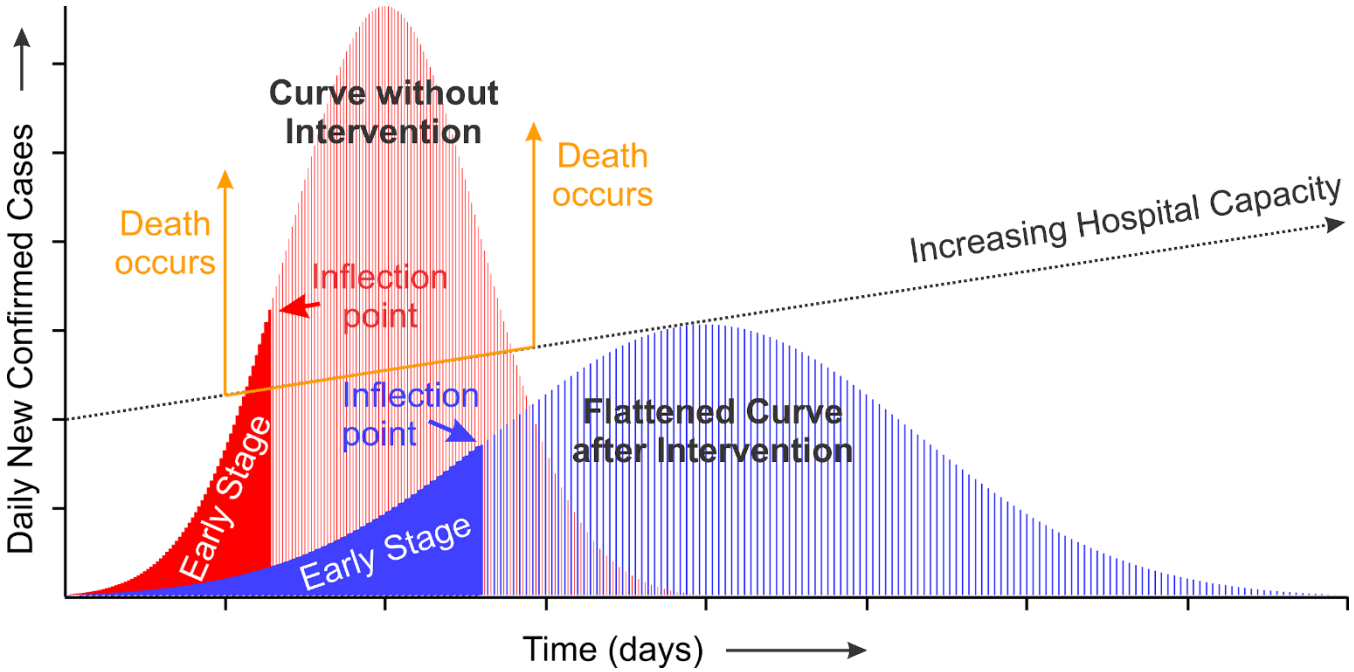

FIGURE 3. Intervention of the COVID-19 outbreak. The intervention at an early stage (before the inflection point, which is the point where the half width of a Gaussian peak is equivalent to the sigma of the Gaussian) of a viral breakout is the key to slow down the transmission of the virus. It not only decreases the peak value of newly confirmed daily cases, but also saves the time to increase the hospital capacity, each of which reduces the overall mortality rate.

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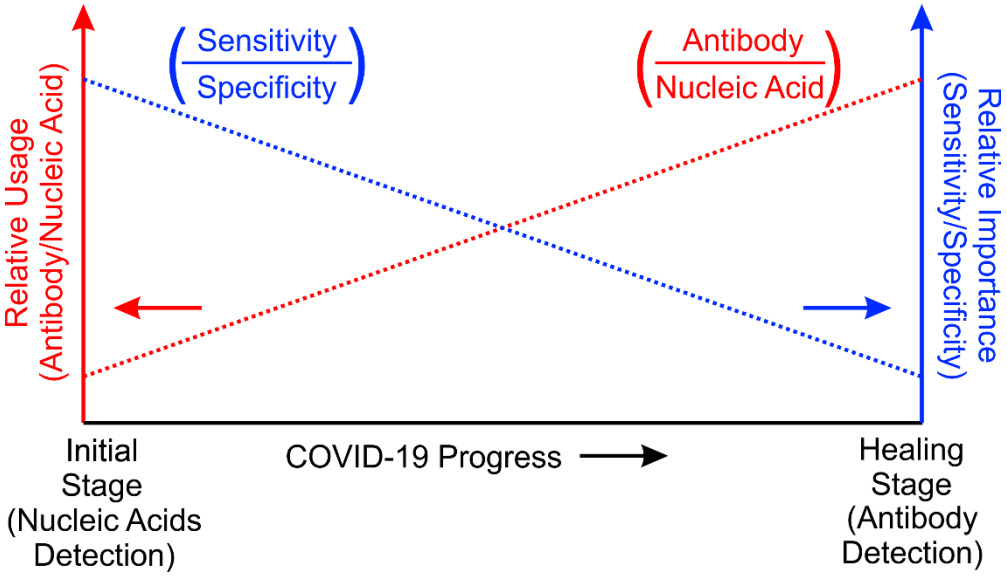

FIGURE 4. Schematic diagram of the relative usage of nucleic acid vs antibody detection methods (left y axis) and the relative importance of sensitivity vs specificity (right y axis) in the detection of SARS-CoV-2 virus during the COVID-19 pandemic. At the initial breakout stage, nucleic acid-based detection methods are important because of their high sensitivity with low false negativity. It allows quick isolation of infected individuals for timely treatment and disease containment. At the healing stage when a society is set to reopen, antibody detection methods are important to identify individuals immune to the disease due to previous COVID-19 infections. Highly specific immunoassays with low false positivity are desirable to correctly identify these individuals who are safe to return to the society. Interestingly, the same pattern can be used to describe individual cases of COVID-19 infections.

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Table 1: Kits based on nucleic acid detection

Authorization	Manufacturer	Mechanism	Target	LOD	Time
05/11/2020*	1drop Inc.	RT-PCR	E, RdRP	200 cp/mL	-
12/17/2020*	3B BlackBio Biotech India Ltd	RT-PCR	RdRP, E, N	10 cp/µL	-
03/2020#	3D Medicines	RT-PCR	-	-	-
€	A*Star Tan Tock Seng Hospital of Singapore	RT-PCR	-	-	-
09/17/2020*	Abbott Diagnostics Scarborough, Inc.	RT-LAMP	RdRP	125 GE/mL	5-13 min
07/30/2020*	Abbott Molecular	RT-PCR	RdRP, N	100 cp/mL	-
12/23/2020*	Abbott Molecular Inc.	RT-PCR	RdRP, N	100 cp/mL	-
07/07/2020*	Access Bio, Inc	RT-PCR	RdRP, N	10 cp/rxn	-
09/25/2020*	Access Genetics, LLC	RT-PCR	RNaseP	15 cp/µL	-
09/24/2020*	Accupath Laboratories, Inc.	RT-PCR	ORF1ab, N, S	25 cp/µl	-
09/30/2020*	Aeon Global Health	RT-PCR	ORF1ab, N, S	0.25 cp/µl	-
11/30/2020*	Agena Bioscience, Inc.	RT-PCR, chip array and MALDI-TOF Mass Spec.	ORF1(ab), N (1,2,3)	2.5 cp/µl	-
09/29/2020*	Akron Children's Hospital	RT-PCR	E, S	250 cp/mL	-
09/30/2020*	Alimetrix, Inc.	RT-PCR, Microarray Hybridization	ORF1ab, N1, N2	250 cp/mL	-
08/10/2020*	Alpha Genomix Laboratories	RT-PCR	ORF1ab, N, S	4 cp/µL	-
04/22/2020*	Altona Diagnostics GmbH	RT-PCR	N, S	0.1 PFU/mL	-
02/2020#	Anatolia Geneworks	RT-PCR	-	-	-
12/07/2020*	Applied BioCode, Inc.	RT-PCR	N	$1.72x10^{-2}$ $TCID_{50}$/mL	-
11/21/2020*	Applied DNA Sciences, Inc.	RT-PCR	RNaseP	5 cp/rxn	-
**	ARUP Laboratories	RT-PCR	-	-	-
09/21/2020*	Assurance Scientific Laboratories	RT-PCR	N1, N2, RNaseP	37 cp/rxn	-
12/28/2020*	Atila BioSystems, Inc	RT-LAMP	N, ORF1ab	4 cp/µL	-
03/2020#	AUSDiagnostics	RT-PCR	-	-	-
09/25/2020*	Avellino Lab USA, Inc.	RT-PCR	RNase P (RP)	55 cp/µL	1-2 days
08/31/2020*	BayCare Laboratories, LLC	RT-PCR	-	-	-
09/23/2020*	Becton, Dickinson & Company	RT-PCR	N, RP	40 GE/mL	-
09/22/2020*	Becton, Dickinson and Company	RT-PCR	N (N1, N2)	40 GE/mL	-
10/31/2020*	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	RT-PCR	ORF1ab, N	100 cp/mL	-
-	Beijing Applied	RT-PCR	ORF1ab, N, E	1000 cp/mL	90 min
04/24/2020*	BGI Genomics Co. Ltd.	RT-PCR	ORF1ab	100 cp/mL	-
-	BGI Wuhan Biotech Co., Ltd	RT-PCR	ORF1ab	100 cp/mL	90 min
09/04/2020*	BillionToOne, Inc.	Sequencing	-	3200 cp/mL	-
12/09/2020*	Bio-Rad Laboratories, Inc.	RT-droplet PCR	N	400 c p/mL	-
12/18/2020*	BioCore Co., Ltd.	RT-PCR	N, RdRp	500 cp/mL	-
12/09/2020*	Bioeksen R&D Technologies Ltd.	RT-PCR	ORF1ab, RNaseP	200 cp/mL	-
12/04/2020*	BioFire Defense, LLC	RT Nested multiplex PCR	ORF1ab, ORF8	330 cp/mL	50 min
12/30/2020*	Biomeme, Inc.	RT-PCR	ORF1ab, S	1.8 GE/µL	-
11/06/2020*	BioMérieux SA	RT-PCR	N, RdRp, E	300 GE/mL	-
-	Bioneer	RT-PCR	-	-	-

**	BioReference Laboratories	RT-PCR	-	-	-
12/28/2020*	BioSewoom, Inc.	RT-PCR	RdRP, E	6.25 cp/µl	-
09/21/2020*	Boston Heart Diagnostics	RT-PCR	N, S, ORF1ab	250 cp/mL	-
07/10/2020*	Boston Medical Center	RT-PCR	N	10 cp/µL	-
2/4/2020*	Centers for Disease Control and Prevention's (CDC)	RT-PCR	N1, N2, RP	4-10 cp/µL	-
07/01/2020*	CENTOGENE US, LLC	RT-PCR	E, RdRP	5 cp/µL	-
3/20/2020*	Cepheid	RT-PCR	N2, E	250 cp/mL	45 min
09/21/2020*	ChromaCode Inc.	RT-PCR	N1, N2	500 cp/mL	-
12/28/2020*	Clear Labs, Inc.	RT-PCR and Sequencing	-	-	-
03/2020	CerTest BioTec	RT-PCR	-	-	-
08/03/2020*	Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute	RT-PCR	E, RdRP	10 cp/µl	-
07/30/2020*	Clinical Reference Laboratory, Inc.	RT-PCR	-	0.25 cp/µl	-
12/18/2020*	Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard	RT-PCR	N1, N2	4 cp/µl	-
4/3/2020*	Co-Diagnostics, Inc	RT-PCR	RdRP	600 cp/spl	-
11/02/2020*	Color Genomics, Inc.	RT-LAMP	N, E, ORF1ab	0.75 cp/µL	-
07/13/2020*	Compass Laboratory Services, LLC	RT-PCR	ORF1ab	5 cp/µL	-
08/20/2020*	Cue Health Inc.	Isothermal amplification	N	1.3 cp/µL	-
10/02/2020*	Cuur Diagnostics	RT-PCR	ORF1ab, N, S	25 cp/µL	-
03/2020#	Credo Diagnostics Biomedical	RT-PCR	-	-	-
-	Daan Gene Co., Ltd., Sun Yat-sen University	RT-PCR	ORF1ab, N	500 cp/mL	110 min
05/22/2020*	Dba SpectronRx	RT-PCR	N, E	5 cp/rxn	-
10/06/2020*	Detectachem Inc.	RT-LAMP	N, E	75 cp/mL	-
10/26/2020*	DiaCarta, Inc	RT-PCR	E, N, ORF1ab	100 cp/mL	-
06/25/2020*	Diagnostic Solutions Laboratory, LLC	RT-PCR	N, S	10 cp/swab	-
03/19/2020*	DiaSorin Molecular LLC	RT-PCR	ORF1ab, S	500 cp/mL	1-1.5 h
12/09/2020*	DxTerity Diagnostics, Inc.	RT-PCR	N, S, ORF1a	150 cp/mL	-
09/21/2020*	Eli Lilly and Company	RT-PCR	N1, N2	100 cp/µL	-
12/30/2020*	Enzo Life Sciences, Inc.	RT-PCR	N1, N2	280 cp/mL	-
09/21/2020*	Ethos Laboratories	RT-PCR and MALDI-TOF Mass Spec.	N, ORF1ab	1 $TCID_{50}$/mL	-
11/04/2020*	Euroimmun US, Inc.	RT-PCR	N, ORF1ab	150 cp/mL	-
09/21/2020*	Exact Sciences Laboratories	RT-PCR	N	1.2 cp/µL	-
11/19/2020*	Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory	RT-PCR	ORF1ab, N, S	160-800 cp/mL	-
07/09/2020*	Fast Track Diagnostics Luxembourg S.á.r.l. (a Siemens Healthineers Company)	RT-PCR	N	0.0023 $TCID_{50}$/mL	-
11/05/2020*	Fluidigm Corporation	RT-PCR	N1, N2	6.25 GE/µL	-
05/15/2020*	Flugent Therapeutics, LLC	RT-PCR	N1, N2, RNaseP	5 cp/mL	-
12/28/2020*	Fosun Pharma USA Inc.	RT-PCR	ORF1ab, N, E	300 cp/mL	-
**	Fulgent Genetics/MedScan laboratory	Sequencing	-	-	-
12/28/2020*	Gencurix, Inc.	RT-PCR	N1, N2	6.25 cp/µL	-
12/28/2020*	Gene By Gene	RT-PCR	RdRP, N	50 cp/rxn	-
05/14/2020*	GeneMatric, Inc.	RT-PCR	RdRp, N	50 cp/rxn	-

10/17/2020*	Genetrack Biolabs, Inc.	RT-PCR	N1, N2	1 cp/µL	-
06/05/2020*	Genetron Health (Beijing) Co., Ltd.	RT-PCR	ORF1ab, N	1000 cp/mL	-
03/2020#	Genetic Signatures	RT-PCR	-	-	-
10/07/2020*	GenMark Diagnostics, Inc.	RT-PCR, electrowetting and sensing	-	100 cp/µL	2 h
03/2020#	Genomica/PharmMar Group	RT-PCR	-	-	-
4/16/2020*	GenoSensor, LLC	RT-PCR	E, N, ORF1ab	1 cp/µL	-
05/08/2020*	Gnomegen LLC	RT-PCR	N1, N2	10 cp/rxn	-
06/01/2020*	Gravity Diagnostics, LLC	RT-PCR	N1, N2, RNaseP	4.8 cp/µL	-
05/14/2020*	Holigic, Inc.	Transcription Mediated Amplification	ORF1ab	0.01 $TCID_{50}$/mL	-
3/16/2020*	Hologic, Inc.	RT-PCR	-	10^{-2} $TCID_{50}$/mL	-
10/28/2020*	Illumina, Inc.	Sequencing	-	-	-
12/28/2020*	InBios International, Inc	RT-PCR	E, N, ORF1ab	12.5 GE/rxn	-
*	Integrated DNA technologies/Danaher	RT-PCR	-	-	-
4/1/2020*	Ipsum Diagnostics, LLC	RT-PCR	N, RP	8.5 cp/µL	-
12/30/2020*	Jiangsu Bioperfectus Technologies Co., Ltd.	RT-PCR	ORF1ab, N	350 cp/mL	-
07/24/2020*	Jiangsu CoWin Biotech Co., Ltd.	RT-PCR	ORF1ab, N	300 cp/mL	-
€	JN Medsys	RT-PCR	-	-	-
09/09/2020*	Kaiser Permanente Mid-Atlantic States	RT-PCR	N, S, ORF1ab	-	-
09/21/2020*	KimForest Enterprise Co., Ltd.	RT-PCR	RdRP	200 cp/mL	-
09/21/2020*	KogeneBiotech Co., Ltd.	RT-PCR	E, RdRP	4 cp/µL	-
06/11/2020*	KorvaLabs, Inc	RT-PCR	N1, N2, RP	200 cp/rxn	-
09/22/2020*	LabGenomics Co., Ltd.	RT-PCR	RdRp, E	20 GE/mL	-
12/09/2020*	Laboratory Corporation of America (LabCorp)	RT-PCR	Rnase P (RP), N	6.25 cp/µL	-
*	LGC, Biosearch Technologies	RT-PCR	-	-	-
06/29/2020*	LifeHope Labs	RT-PCR	N1, N2	2.5 GE/µL	
11/17/2020*	Lucira Health, Inc.	RT-LAMP	-	900 cp/mL	30 min
10/28/2020*	Luminex Corporation	RT-PCR	ORF1ab, N	75 GE/µL	-
10/28/2020*	Luminex Molecular Diagnostics, Inc.	RT-PCR	ORF1ab, N gene, E	1.5 cp/µL	4 h
4/15/2020*	Maccura Biotechnology (USA) LLC	RT-PCR	E, N, ORF1ab	1 cp/µL	2 h
3/23/2020*	Mesa Biotech Inc.	RT-PCR and colorimetry	N	100 cp/rxn	30 min
3/30/2020*	NeuMoDx Molecular	RT-PCR	-	-	-
3/30/2020*	NeuMoDx Molecular, Inc.	RT-PCR	Nsp2, N	150 cp/mL	
3/20/2020*	Novacyt/Primerdesign	RT-PCR	-	-	-
05/06/2020*	OPTI Medical Systems, Inc.	RT-PCR	N1, N2	0.7 cp/µL	-
04/18/2020*	OSANG Healthcare	RT-PCR	RdRp, N, E	0.5 cp/µL	-
02/2020#	OsangHealthCare	RT-PCR	-	-	-
3/24/2020*	PerkinElmer, Inc.	RT-PCR	ORF1ab, N	20 cp/mL	-
06/04/2020*	Phosphorus Diagnostics LLC	RT-qPCR	N1, N2, RNaseP	5 cp/µL	-
3/20/2020*	Primerdesign Ltd.	RT-PCR	-	0.33 cp/µL	-
3/30/2020*	QIAGEN GmbH	RT-PCR	-	500 cp/mL	-
3/17/2020*	Quest Diagnostics Infectious Disease, Inc.	RT-PCR	N1, N3	136 cp/mL	-
3/23/2020*, 3/2020#	Quidel Corporation	RT-PCR	Pp1ab	0.8 cp/µL	75 min

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05/18/2020*	Quidel Corporation	RT-PCR	Pp1ab	$1.28x10^4$ Genome eq/mL	-
03/2020$	Rendu Biotechnology	RT-LAMP	-	-	-
04/29/2020*	Rheonix, Inc.	Endpoint RT-PCR	N1	625 GE/mL	-
3/12/2020*	Roche Molecular Systems, Inc.	RT-PCR	E	-	3 hrs
-	SANSURE Bio-tech Co., Ltd	RT-PCR	ORF1ab, N	200 cp/mL	90 min
05/04/2020*	Sansure BioTech Inc.	RT-PCR	ORF1ab, N	200 cp/mL	-
4/3/3030*	ScienCell Research Laboratories	RT-PCR	N (N1, N2)	500 cp/µL	-
04/23/2020*	SD Biosensor, Inc	RT-PCR	ORF1ab, RdRp, E	0.5 cp/µL	-
04/27/2020*	SEASUN BIOMATERIALS	RT-PCR	ORF1ab, N	1 cp/µL	-
05/21/2020*	Seasun Biomaterials, Inc.	RT-LAMP	ORF1ab, RNaseP	-	-
04/21/2020*	Seegene, Inc.	RT-PCR	E, RdRp, N	4167 cp/mL	-
-	Shanghai Bio Germ	RT-PCR	ORF1ab, N	1000 cp/mL	90 min
-	Shanghai GeneoDx Biotech Co., Ltd	RT-PCR	ORF1ab, N	500 cp/mL	90 min
-	Shanghai ZJ Bio-tech Co., Ltd.	RT-PCR	ORF1ab, N, E	1000 cp/mL	90 min
05/06/2020*	Sherlock BioSciences, Inc.	CRISPR	ORF1ab, N, RNaseP	1-4.5 cp/µL	-
2/2020#, $$, $$$	SolGent	RT-PCR	-	-	-
05/21/2020*	SolGent Co., Ltd.	RT-PCR	N, ORF1	200 cp/mL	-
03/2020#	Systaaq Diagnostic Products	RT-PCR	-	-	-
3/13/2020*	Thermo Fisher Scientific, Inc.	RT-PCR	S, N	10 GE/rxn	4 h
03/2020#	TIB MolBiol Synthesalabor	RT-PCR	E	-	-
04/20/2020*	Trax Management Services Inc.	RT-PCR	RNaseP	50 cp/mL	-
-	Ustar	RT-PCR	ORF1ab, N	-	90 min
03/2020#	Vision Medicals	PCR (Clinical Sequencing Assay)	-	-	-
2/29/2020*	Wadsworth Center, New York State Department of Public Health's (CDC)	RT-PCR	RP	25 cp/rxn	-
-	Wuhan Easydiagnosis	RT-PCR	ORF1ab, N	-	75 min

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Table 2: Immunoassay kits for blood antibody tests (**Ig**) or viral antigen test (**Ag**)

Authorization	Manufacturer	Mechanism	Target	LOD	Time
12/16/2020*	Abbott Diagnostics Scarborough, Inc.	Lateral flow assay	N protein (**Ag**)	140.6 $TCID_{50}$/mL	15 min
12/01/2020 *	Abbott Laboratories, Inc.	Chemiluminescent micro-particle immunoassay	IgG, IgM	-	-
10/13/2020*	Access Bio, Inc.	Lateral flow assay	N protein (**Ag**)	800 $TCID_{50}$/ml.	10 min
10/31/2020*	Access Bio, Inc.	Lateral flow assay	IgG, IgM	100% sensitivity, 97.5% specificity	-
12/15/2020*	ACON Laboratories, Inc.	Lateral flow assay	IgG, IgM	100% sensitivity, 96.2% specificity	-
09/23/2020*	Assure Tech. (Hangzhou Co., Ltd)	Lateral flow assay	IgG, IgM	-	15 min
04/24/2020*	Autobio Diagnostics Co. Ltd.	Lateral Flow Immunoassay	IgG, IgM	-	50 min
06/23/2020*	Babson Diagnostics, Inc.	Chemiluminescence immu-noassay	IgG	-	-
10/08/2020*	Beckman Coulter, Inc.	Chemiluminescence immu-noassay	IgG, IgM	-	-
07/23/2020*	Becton, Dickinson and Company (BD)	Chromatographic Digital Immunoassay	N protein (**Ag**)	140 $TCID_{50}$/mL	15 min
***	Beijing Decombio Biotechnology	Immunoassay	IgG, IgM	-	-
***	Beijing Diagreat Biotechnologies	Immunoassay	IgG, IgM	-	-
***	Beijing Kewei Clinical Diagnostic Reagent	Immunoassay	IgG, IgM	-	-
***	Beijing O&D Biotech	Colloidal gold	-	-	-
08/05/2020*	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	ELISA	Total antibody	96.7% sensitivity, 97.5% specificity	~ 1 h
***	Beroni Group	Immunoassay	IgG, IgM	-	-
08/25/2020*	Biocan Diagnostics Inc.	Lateral flow assay	IgG, IgM	100% sensitivity, 99.4% specificity	-
08/17/2020*	BioCheck, Inc.	Chemiluminescence immu-noassay	IgG, IgM	-	-
10/17/2020*	Biohit Healthcare (Hefei) Co. Ltd.	Lateral flow assay	IgG, IgM	96.7% sensitivity, 95% specificity	-
***	BioMedomics	Immunoassay	IgG, IgM	-	-
08/06/2020*	bioMérieux SA	Enzyme-linked fluores-cence assay	IgG, IgM	-	-
10/30/2020*	Bio-Rad Laboratories, Inc.	ELISA	Total antibody	98% sensitivity, 99.3% specificity	-
-	BiOSCiENCE	Immunoassay	IgM, IgG	-	30 min
***	BTNX	Immunoassay	IgG, IgM	-	-
06/12/2020*	Cellex Inc.	Lateral flow assay	IgG, IgM	-	20 min
10/23/2020*	Celltrion USA, Inc.	Magnetic Force-assisted Electrochemical Sandwich Immunoassay (MESIA)	S protein (**Ag**)	30 $TCID_{50}$/mL	-
4/14/2020*	Chembio Diagnostic System, Inc	Immunoassay	IgG, IgM	-	-
***	Core Technology	Immunoassay	IgG, IgM	-	-
10/19/2020*	DiaSorin Inc.	Chemiluminescence immu-noassay	IgG, IgM	-	-
08/17/2020*	Diazyme Laboratories, Inc.	Chemiluminescence immu-noassay	IgG, IgM	-	-
***	Eachy Biopharmaceuticals	Immunoassay	IgG, IgM	-	-
-	Eagle Bioscience	Immunoassay	IgG, IgM	-	-
12/23/2020*	Ellume Limited	Lateral Flow Fluorescence assay	N protein (**Ag**)	103.80 $TCID_{50}$/mL	-
06/15/2020*	Emory Medical Laboratories	ELISA	IgG	-	-
05/04/2020*	EUROIMMUN US Inc.	ELISA	IgG	90% sensitivity, 100% specificity	2.5 h
10/08/2020*	Genalyte, Inc.	Photonic ring immunoas-say	Total antibody	-	-
11/06/2020*	GenScript USA Inc.	Blocking ELISA	Total Neutral-izing Antibod-ies	-	-

-	Guangdong Hecin	Immunoassay	IgM	-	-
***	Guangzhou Wondfo	Immunoassay	-	-	15 min
***	Hangzhou AllTest Biotech	Immunoassay	IgG, IgM	-	-
12/21/2020*	Hangzhou Biotest Biotech Co., Ltd.	Lateral flow assay	IgG, IgM	100% sensitivity, 100% specificity	10 min
***	Hangzhou Clongene Biotech	Immunoassay	IgG, IgM	-	-
12/02/2020*	Hangzhou Laihe Biotech Co., Ltd.	Lateral flow assay	IgG, IgM	100% sensitivity, 98.8% specificity	-
***	Hangzhou Testsealabs Biotech-nology	Immunoassay	IgG, IgM	-	-
05/29/2020*	Healgen Scientific LLC	Lateral flow assay	IgG, IgM	100% sensitivity, 97.5% specificity	10 min
06/30/2020*	InBios International, Inc.	ELISA	IgG	96.7% sensitivity, 98.8% specificity	-
06/10/2020*	InBios International, Inc.	ELISA	IgM	100% sensitivity, 100% specificity	-
11/23/2020*	Innovita (Tangshan) Biological Technology Co., Ltd.	Lateral flow assay	IgG, IgM	100% sensitivity, 97.5% specificity	15 min
***	Jiangsu Macro & Micro-Test Med-Tech	Colloidal gold	IgG, IgM	-	-
11/10/2020*	Jiangsu Well Biotech Co., Ltd.	Lateral flow assay	IgG, IgM	97.14% sensitivity, 100% specificity	-
11/24/2020*	Kantaro Biosciences, LLC	ELISA	IgG	-	-
***	Lifeassay Diagnostics	Immunoassay	IgM, IgG	-	-
07/16/2020*	Luminex Corporation	Fluorescent microsphere Immunoassay	IgG	-	-
12/07/2020*	Luminostics, Inc.	Lateral flow immunolumi-nescent assay	N protein (**Ag**)	88 $TCID_{50}$/mL	30 min
08/18/2020*	LumiraDx UK Ltd.	Microfluidic Immunofluo-rescence Assay	N protein (**Ag**)	32 $TCID_{50}$/mL	12 min
***	Medical Systems Biotechnology	Immunoassay	IgG, IgM	-	-
07/17/2020*	Megna Health, Inc.	Lateral flow assay	IgG, IgM	100% sensitivity, 95% specificity	-
04/15/2020*	Mount Sinai Laboratory	ELISA	IgG	-	-
***	Nanjing Liming Bio-Products	Immunoassay	IgG, IgM	-	-
-	Nanjing Vazyme	Immunoassay	IgM, IgG	-	10 min
09/29/2020*	NanoEntek America, Inc.	Fluorescence immunoas-say	Total antibody	96.7% sensitivity, 98.8% specificity	-
***	NanoResearch	Immunoassay	IgG, IgM	-	-
***	Nantong Diagnos Biotechnology	Colloidal gold	-	-	-
12/31/2020*	Nirmidas Biotech, Inc.	Lateral flow assay	IgG, IgM	100% sensitivity, 96.2% specificity	-
10/30/2020*	Ortho Clinical Diagnostics, Inc.	Chemiluminescence immu-noassay	Total antibody	-	-
10/23/2020*	Ortho-Clinical Diagnostics, Inc.	Chemiluminescence immu-noassay	IgG, IgM	-	10-15 min
***	PCL	Immunoassay	IgG, IgM	-	-
***	Promedical	Lateral Flow Immunoassay	-	-	-
***	PharmaTech	Immunoassay	IgG, IgM	-	-
10/28/2020*	Quansys Biosciences, Inc.	Chemiluminescence immu-noassay	IgG	-	-
12/23/2020*	Quanterix Corporation	Paramagnetic Microbead-based Sandwich ELISA	IgG	-	-
12/22/2020*	Quidel Corporation	Lateral flow assay	N protein (**Ag**)	7570 $TCID_{50}$/mL	10 min
09/25/2020*	Quotient Suisse SA	Photometric immunoassay	Total antibody	-	-
10/23/2020*	Roche Diagnostics	Electrochemiluminescence immunoassay	Total antibody	-	18 min
07/13/2020*	Salofa Oy	Lateral flow assay	IgG, IgM	93.3% sensitivity, 98.8% specificity	-
***	SD Biosensor	Immunoassay	IgG, IgM	-	-
***	Shenzhen Landwind Medical	Immunoassay	IgG, IgM	-	-

09/14/2020*	Shenzhen New Industries Biomedical Engineering Co., Ltd.	Chemiluminescence immunoassay	IgG, IgM	-	-
10/30/2020*	Siemens Healthcare Diagnostics Inc.	Chemiluminescence immunoassay	Total antibody	-	10 min
02/2020#	Snibe Diagnostics	Immunoassay	IgG, IgM	-	-
09/03/2020*	Sugentech, Inc.	Lateral flow assay	IgG	96.7% sensitivity, 100% specificity	-
08/31/2020*	TBG Biotechnology Corp.	Lateral flow assay	IgG, IgM	93.3% sensitivity, 95% specificity	-
***	Telepoint Medical Services	Immunoassay	IgG, IgM	-	-
10/06/2020*	Thermo Fisher Scientific	ELISA	Total antibody	96.7% sensitivity, 97.5% specificity	-
***	Tianjin Beroni Biotechnology	Immunoassay	IgG, IgM	-	-
08/31/2020*	University of Arizona Genetics Core for Clinical Services	ELISA	Total antibody	-	-
06/04/2020*	Vibrant America Clinical Labs	Chemiluminescence immunoassay	IgG, IgM	-	-
04/30/2020*	Wadsworth Center, New York State Department of Health	Fluorescent microsphere Immunoassay	Total antibody	-	-
11/06/2020*	Xiamen Biotime Biotechnology Co., Ltd.	Lateral flow assay	IgG, IgM	100% sensitivity, 96.2% specificity	-
-	Xiamen innoDx Bio-tech	Immunoassay	IgG, IgM	-	-
10/06/2020*	ZEUS Scientific, Inc.	ELISA	IgG	93.3% sensitivity, 100% specificity	
***	Zuhai Livzon Diagnostics	Colloidal gold	IgG, IgM	-	15 min

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*US EUA Authorized, **US EUA Planned, ***US Notified FDA under section IV.D, ****US EUA Submitted, #European Union Conformity Marked, $The National Medical Product Administration Authorized China, $$Korea Ministry of Food and Drug Safety, $$$Philippines Food and Drug Administration, €Singapore Health Sciences Authority, personal authorization for clinical use, λEUA India, ψKorea Centers for Disease Control and the Korea Food and Drug Administration
-Data Not Available
References for kits in Table 1&2:
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