Detecting the Coronavirus (COVID-19)

Pravin Pokhrel[Ψ], Changpeng Hu[Ψ], and Hanbin Mao*

Department of Chemistry and Biochemistry, Kent State University, Kent, OH, USA (44240)

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 July 2020, more than one hundred kits for the COVID-19 diagnosis on the market are surveyed in this review, while emerging sensing techniques for the 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 the society is set to reopen from the lock-down stage of the COVID-19 pandemic, it becomes critical to have immunoassay based kits with high specificity to identify people who can safely return to the society after their recovery of SARS-CoV-2 infections. 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 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.

Background

Since the beginning of the 21st century, our world has been facing unprecedented crises of deadly viruses like Zika, Ebola, SARS, MERS, and so forth. The epidemics of these viral diseases were sparked either by the evolution of pre-existing viruses or by the emergence of new viral species. Such 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]

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 the 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 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.

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. Finally, we wrap up by proposing the 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.

The COVID-19 outbreak

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.

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].

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 membrane[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].

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 an early stage and immediately isolate infected patients. Currently, there are three methods in clinical practice to diagnose COVID-19, which are summarized below.

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 *Nucleic acid based methods* below) showed that CT imaging is 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.

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.

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, Nucleocapsid (N) gene, E gene, Spike protein (S gene), and ORF1ab gene. 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. 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 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, 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 varies with the specimen types. To et al. revealed that the 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 suggest 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[33] provides a convenient way to develop affordable point-of-care testing kits that can be massively used by the general public (see Sections *Ideal characteristics of diagnostic methods* and *Emerging techniques to detect SARS-CoV-2* below).

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

Immunoassays. Immunoassay is another established diagnostic method. This method detects viral protein antigens or serum antibodies in patients who have been exposed to the SARS-CoV-2. These antibody tests are important in detecting prior infections.

In the SARS-CoV-2 infection, studies have shown that the seroconversion in the patient-generally starts after a week of the first symptom[34]. In a study of post symptomatic patients, Amanat et al. detected high IgA and IgM immune responses[35]. 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%[36]. 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[37]. In a comparative study, both ELISA and colloidal gold immunochromatographic kits showed equal sensitivity with 100% specificity for the SARS-CoV-2 detection[38].

Several immunoassay kits are already on market for emergency detection of COVID-19 specific antibodies (see Table 2). However, the major problem of this method is that it only works for post-symptomatic 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[39], potential cross reactivity (less specific)[40], and low sensitivity with respect to nucleic-acid based methods. Nevertheless, immunoassays are faster[41] and cheaper than the RT-PCR methods. They 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.

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[42]. 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 kits available 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)[43] 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 testing kits such as lateral flow immunoassays[44] 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 Rapid HIV test[45] in which HIV infection can be quickly determined at home using paper strips on specimens such as blood samples. Due to the requirements of easy usage and cheap price, they often use colloidal gold based immunoassay mechanisms. Such POC testing kits perhaps represent the best solution to fight fast transmitting pandemics.

Emerging techniques to detect SARS-CoV-2

Given a variety of problems associated with current clinical diagnosis for the SARS-Cov-2 (Section *Diagnosis of the COVID-19*), here, we discuss some promising techniques that may address these issues.

Isothermal amplification for nucleic acid targets. Although RT-PCR is a widely used method in the confirmatory screening of COVID-19 infections (see Section *Nucleic acid based methods*), it is time consuming and requires sophisticated laboratory facility and trained personal to operate[46]. 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 $^{\circ}$C[47]. 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[48–51]. As a result, the target sequence is amplified exponentially, which is detected by turbidimetry[52] or fluorescence[53]/colorimetry[48].

As an example, the RNA extraction and LAMP amplification have been performed in the same tube[51,54]. 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[55]. 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[56] and Chiu group[57] integrated the LAMP with the CRISPR-based SHERLOCK (see Table 1)[58] and CRISPR-Cas12 based 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 [59]. This may be attributed to the compromised performance of the RdRP target[60,61] used in this assay, which is found to be mutating and evolving[62].

Rolling circle amplification (RCA)[63] 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[64]. 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 offers high sensitivity comparable to RT-PCR[65] since it amplifies the target sequence by ~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[65]. 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.

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 papers with immobilized capture probes. Upon binding with nucleic acid targets, the probes give a visible signal[66–68]. 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.[68] 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[68]. 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[69].

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, IgM/IgG 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[66]. 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[70]. Other signal amplification strategies include solvent evaporation for analyte preconcentrations, nanoparticle catalyzed nanoparticle labeled assays, and ion concentration polarization methods[71].

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[72]. These cameras are sensitive to subtle color changes and hence provide more effective color detection than traditional RGB sensors or the naked eye[73]. For improved read-out of the results and data processing, machine learning algorithm could also be used[74]. Smartphones can also be coupled with external adapters to integrate external biosensor platforms for more versatile POC testing[75].

Other emerging methods. As discussed in section *Ideal characteristics of diagnostic methods*, 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 *Isothermal amplification for nucleic acid targets* and *Lateral flow based detection of nucleic acid targets*, other emerging methods have been developed to improve these features. To improve the sensitivity, methods with single-molecule detection capability can be used[76–78]. As an example, the single molecule enzyme linked immunosorbent assay (ELISA) has been developed to offer detection limit of subfemtomolar protein concentrations[79]. 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[80]. 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[81] and DNA microarray[82] can be used. In the case of COVID-19, evidences 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[83]. The DNA microarray has been used in high-throughput identification of mutations in SARS-CoV-2[84]. 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 cost, therefore, they may not be appropriate for the economic and rapid screening in the COVID-19 pandemic.

Rationales in choosing diagnostic methods in the COVID-19 outbreak

As stated in the introduction, 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 challenge. 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 1-3). 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[85,86] 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 identification. 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[87,88]. 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[89] (see Table 1) usually have superior sensitivities albeit with increased false positive levels[87,88] (see Section *Isothermal amplification for nucleic acid targets*). 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 *Chest CT imaging*, 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.

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 one week. Since these immunoassays are cheaper, faster, and easier to perform[44] with respect to nucleic acid based methods, they can be quickly and massively conducted by staff at drive-thru stations for example. 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).

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 immunoassay based 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.

In the future, affordable POC testing (POCT) kits as discussed in section *Ideal characteristics of diagnostic methods* may present a viable direction to address the bottleneck diagnosis problem caused by shortage of testing kits. These kits can be performed at home for self-isolated people with mild symptoms. If they are tested positively 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 pandemic. We therefore name such an approach a massive POCT strategy. Given there is no such massive POCT product on the market for the COVID-19 diagnosis yet, research and development on the affordable POCT kits are dearly needed at this stage for virus detections.

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. 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 level (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 immunoassays is especially useful at the later stages of the virus outbreak when people who have been recovered from the COVID-19 are identified for their reengagement to the society. 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.

Vocabulary

RT-PCR: A type of polymerase chain reaction to detect target RNA.

Sensitivity: In diagnostics, it is the measure of how accurately a true positive case is identified positive. Also, it gives the measure of lowest concentration of a target that can be detected with that diagnostic method.

Specificity: In diagnostics, it is the measure of how accurately a true negative case is identified negative.

POCT (Point-of-care Testing) – Tests that give an immediate result to make onsite and informed decisions about patient care.

False positive: True negative cases identified as positive. This is the consequence of low specificity.

False negative: True positive cases identified as negative. This is the consequence of low sensitivity.

AUTHOR INFORMATION

Corresponding Author

*Hanbin Mao, PhD
Department of Chemistry and Biochemistry, Kent State University, OH, USA
hmao@kent.edu; (+1) 330-672 9380
850 University Esplanade, Kent, OH, 44242-0001, P.O. Box 5190
ORCID: 0000-0002-6720-9429

Author Contributions

Ψ These authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

REFERENCES

1. Baud, D.; Qi, X.; Nielsen-Saines, K.; Favre, G. Real estimates of mortality following COVID-19 infection. *Lancet Infect. Dis.* (2020). DOI: 10.1016/ S1473-3099(20)30195-X

2. Cohen, D.; Carter, P. WHO and the pandemic flu "conspiracies". *BMJ* **340**, (2010). DOI: 10.1136/bmj.c2912

3. Wilder-Smith, A.; Chiew, C. J.; Lee, V. J. Can we contain the COVID-19 outbreak with the same measures as for SARS? *Lancet Infect. Dis.* (2020). DOI: 10.1016/S1473-3099(20)30129-8

4. Du, Z.; Xu, X.; Wu, Y.; Wang, L.; Cowling, B. J.; Meyers, L. A. Serial Interval of COVID-19 among Publicly Reported Confirmed Cases - Volume 26, Number 6—June 2020 - *Emerging Infectious Diseases journal - CDC.* DOI:10.3201/eid2606.200357.

5. WHO. Managing epidemics: Key facts about major deadly diseases. (2018) https://www.who.int/publications/i/item/managing-epidemics-key-facts-about-major-deadly-diseases

6. Knobler, S.; Mahmoud, A.; Lemon, S.; Mack, A.; Sivitz, L.; Oberholtzer, K. Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary. *National Academies Press (US)*, (2004). DOI: 10.17226/10915

7. WHO. Novel Coronavirus (2019-nCoV) situation report-1. (January 21, 2020). https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

8. Guo, Y.; Cao, Q.; Hong, Z.; Tan, Y.; Chen, S.; Jin, H.; Tan, K.; Wang, D.; Yan, Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Mil. Med. Res.* **7**, 1–10 (2020). DOI: 10.1186/s40779-020-00240-0

9. Guan, W.; Ni, Z.; Hu, Y.; Liang, W.; Ou, C.; He, J.; Liu, L.; Shan, H.; Lei, C.; Hui, D.S.C. *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* (2020). DOI: 10.1056/NEJMoa2002032

10. Zeng, L. K.; Tao, X. W.; Yuan, W. H.; Wang, J.; Liu, X.; Liu, Z. S. First case of neonate infected with novel coronavirus pneumonia in China. *Zhonghua Er Ke Za Zhi Chin. J. Pediatr.* **58**, E009 (2020). DOI: 10.3760/cma.j.issn.0578-1310.2020.0009

11. Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qui, Y.; Wang, J.; Liu, Y.; Wei, Y. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* **395**, 507–513 (2020). DOI: 10.1016/S0140-6736(20)30211-7

12. Lu, C.; Liu, X.; Jia, Z. 2019-nCoV transmission through the ocular surface must not be ignored. *The Lancet* **395**, e39 (2020). DOI: 10.1016/S0140-6736(20)30313-5

13. Zou, L.; Ruan, F.; Huang, M.; Liang, L.; Huang, H.; Hong, Z.; Yu, J.; Kang, M.; Song, Y.; Xia, J. *et al.* SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med.* **382**, 1177–1179 (2020). DOI: 10.1056/NEJMc2001737

14. Rothe, C.; Schunk, M.; Sothmann, P.; Bretzel, G.; Froeschl, G.; Wallrauch, C.; Zimmer, T.; Thiel, V.; Janke, C.; Guggemos, W. *et al.* Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N. Engl. J. Med.* **382**, 970–971 (2020). DOI: 10.1056/NEJMc2001468

15. Bernheim, A.; Mei, X.; Huang, M.; Yang, Y.; Fayad, Z.A.; Zhang, N.; Diao, K.; Lin, B.; Zhu, X.; Li, K. *et al.* Chest CT findings in coronavirus disease-19 (COVID-19): relationship to duration of infection. *Radiology* 200463 (2020). DOI: 10.1148/radiol.2020200463

16. Kong, W.; Agarwal, P. P. Chest imaging appearance of COVID-19 infection. *Radiol. Cardiothorac. Imaging* **2**, e200028 (2020). DOI: 10.1148/ryct.2020200028

17. Pan, F.; Ye, T.; Sun, P.; Gui, S.; Liang, B.; Li, L.; Zheng, D.; Wang, J.; Hesketh, R. L.; Yang, L. *et al.* Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. *Radiology* 200370 (2020). DOI: 10.1148/radiol.2020200370

18. Shi, H.; Han, X.; Jiang, N.; Cao, Y.; Alwalid, O.; Gu, J.; Fan, Y.; Zheng, C. *et al.* Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect. Dis.* (2020). DOI: 10.1016/S1473-3099(20)30086-4

19. Zu, Z. Y.; Jiang, M. D.; Xu, P. P.; Chen, W.; Ni, Q. Q.; Lu, M. G.; Zhang, L. J. *et al.* Coronavirus disease 2019 (COVID-19): a perspective from China. *Radiology* 200490 (2020). DOI: 10.1148/radiol.2020200490

20. Hosseiny, M.; Kooraki, S.; Gholamrezanezhad, A.; Reddy, S.; Myers, L. Radiology perspective of coronavirus disease 2019 (COVID-19): lessons from severe acute respiratory syndrome and Middle East respiratory syndrome. *Am. J. Roentgenol.* 1–5 (2020). DOI: 10.2214/AJR.20.22969

21. American Roentgen Ray Society. Novel coronavirus (COVID-19) imaging features overlap with SARS and MERS: COVID-19's imaging features are variable and nonspecific, but the imaging findings reported thus far do show. *ScienceDaily* https://www.sciencedaily.com/releases/2020/02/200228142018.htm.

22. Fang, Y.; Zhang, H.; Xie, J.; Lin, M.; Ying L.; Pang, P.; Ji, W. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. *Radiology* 200432 (2020). DOI: 10.1148/radiol.2020200432

23. Ai, T.; Yang, Z.; Hou, H.; Zhan, C.; Chen, C.; Lv, W.; Tao, Q.; Sun, Z.; Xia, L. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology* 200642 (2020). DOI: 10.1148/radiol.2020200642

24. Heymann, D. L.; Shindo, N. COVID-19: what is next for public health? *The Lancet* **395**, 542–545 (2020). DOI: 10.1016/S0140-6736(20)30374-3

25. Bustin, S. A.; Mueller, R. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clin. Sci.* **109**, 365–379 (2005). DOI: 10.1042/CS20050086

26. Bustin, S. A. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J. Mol. Endocrinol.* **29**, 23–39 (2002). DOI: 10.1677/jme.0.0290023

27. Chen, H.; Guo, J.; Wang, C.; Luo, F.; Yu, X.; Zhang, W.; Li, J.; Zhao, D.; Xu, D.; Gong, Q. *et al.* Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *The Lancet* **395**, 809–815 (2020). DOI: 10.1016/S0140-6736(20)30360-3

28. Chu, D. K. W.; Pan, Y.; Cheng, S. M. S.; Hui, K. P. Y.; Krishnan, P.; Liu, Y.; Ng, D. Y. M; Wan, C. K. C.; Yang, P.; Wang, Q. *et al.* Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin. Chem.* **66**, 549–555 (2020). DOI: 10.1093/clinchem/hvaa029

29. WHO. Summary of available protocols to detect SARS-CoV-2. (2020) https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2

30. Wang, C.; Horby, P. W.; Hayden, F. G.; Gao, G. F. A novel coronavirus outbreak of global health concern. *The Lancet* **395**, 470–473 (2020). DOI: 10.1016/S0140-6736(20)30185-9

31. To, K. K.; Tsang, O. T.; Yip, C. C.; Chan, K.; Wu, T.; Chan, J. M. C.; Leung, W.; Chik, T. S.; Choi, C. Y.; Kandamby, D. H. *et al.* Consistent detection of 2019 novel coronavirus in saliva. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* (2020). DOI: 10.1093/cid/ciaa149

32. Yam, W. C.; Chan, K. H.; Poon, L. L. M.; Yuen, K. Y.; Seto, W. H.; Peiris, J. S. M. Evaluation of reverse transcription-PCR assays for rapid diagnosis of severe acute respiratory syndrome associated with a novel coronavirus. *J. Clin. Microbiol.* **41**, 4521–4524 (2003). DOI: 10.1128/jcm.41.10.4521-4524.2003

33. Rutgers Today. New Rutgers Saliva Test for Coronavirus Gets FDA Approval. (April 13, 2020) https://www.rutgers.edu/news/new-rutgers-saliva-test-coronavirus-gets-fda-approval

34. Gao, Y.; Yuan, Y.; Li, T. T.; Wang, W. X.; Li, Y. X.; Li, A.; Han, F. M. Evaluation the auxiliary diagnosis value of antibodies assays for detection of novel coronavirus (SARS-Cov-2) causing an outbreak of pneumonia (COVID-19). *medRxiv* (2020). DOI: 10.1101/2020.03.26.20042044

35. Amanat, F.; Stadbauer, D.; Strohmeier, S.; Nguyen, T. H. O.; Chromikova, V.; McMahon, M.; Jiang, K.; Arunkumar, G. A.; Jurczyszak, D.; Polanco, J. *et al.* A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med* (2020). DOI: 10.1038/s41591-020-0913-5

36. Liu, W.; Liu, L.; Kou, G.; Zheng, Y.; Ding, Y.; Ni, W.; Wang, Q.; Tan, Li,; Wu, W.; Tang, S.; *et al.* Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2. *J. Clin. Microbiol.* (2020). DOI: 10.1128/JCM.00461-20

37. Okba, N. M.; Muller, M. A.; Li, W.; Wang, C.; GeurtsvanKessel, C. H.; Corman, V. M.; Lamers, M. M.; Sikkema, R. S.; Bruin, E.; Chandler, F. D. *et al.* SARS-CoV-2 specific antibody responses in COVID-19 patients. *Emerg Infect Dis* (2020). DOI: 10.3201/eid2607.200841

38. Xiang, J.; Yan, M.; Li, H.; Liu, T.; Lin, C.; Huang, S; Shen, C. Evaluation of Enzyme-Linked Immunoassay and Colloidal Gold-Immunochromatographic Assay Kit for Detection of Novel Coronavirus (SARS-Cov-2) Causing an Outbreak of Pneumonia (COVID-19). *medRxiv* (2020). DOI: 10.1101/2020.02.27.20028787

39. Kassanjee, R.; Pilcher, C. D.; Busch, M. P.; Murphy, G.; Facente, S. N.; Keating, S. M.; Mckinney, E.; Marson, K.; Price, M. A.; Martin, J. N. *et al.* Viral load criteria and threshold optimization to improve HIV incidence assay characteristics-a CEPHIA analysis. *AIDS Lond. Engl.* **30**, 2361 (2016). DOI: 10.1097/QAD.0000000000001209

40. Tate, J.; Ward, G. Interferences in immunoassay. *Clin. Biochem. Rev.* **25**, 105 (2004).

41. Sheridan, C. Fast, portable tests come online to curb coronavirus pandemic. *Nat. Biotechnol.* (2020) DOI:10.1038/d41587-020-00010-2.

42. Peeling, R. W., Holmes, K. K., Mabey, D.; Ronald, A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex. Transm. Infect.* **82**, v1–v6 (2006). DOI: 10.1136/sti.2006.024265

43. US FDA. Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics. *FDA* (2020) https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-new-policy-help-expedite-availability-diagnostics

44. Hesterberg, L. K.; Crosby, M. A. An overview of rapid immunoassays. *Lab. Med.* **27**, 41–46 (1996). DOI: 10.1093/labmed/27.1.41

45. Branson, B. M. Point-of-Care Rapid Tests for HIV Antibodies. *Lab. Med.* **27**, 288–295 (2003). DOI: 10.1046/j.1439-0477.2003.03049.x

46. Bustin, S. A.; Nolan, T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. *J. Biomol. Tech. JBT* **15**, 155 (2004).

47. Silva, S. J. R.; Pardee, K.; Pena, L. Loop-Mediated Isothermal Amplification (LAMP) for the Diagnosis of Zika Virus: A Review. *Viruses* **12**, 19 (2020). DOI: 10.3390/v12010019

48. Zhang, Y.; Odiwuor, N.; Xiong, J.; Sun, L.; Nyaruaba, R. O.; Wei, H.; Tanner, N. A. Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP. *medRxiv* (2020). DOI: 10.1101/2020.02.26.20028373

49. Yu, L.; Wu, S.; Hao, X.; Dong, X.; Mao, L.; Pelechano, V.; Chen, W.; Yin, X. Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. *Clin. Chem.* (2020). DOI: 10.1093/clinchem/hvaa102

50. Park, G.; Ku, K.; Baek, S.; Kim, S.; Kim, S.; Kim, B.; Maeng, J. Development of Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) Assays Targeting SARS-CoV-2. *J. Mol. Diagn.* (2020). DOI: 10.1016/j.jmoldx.2020.03.006

51. Huang, W. E.; Lim, B.; Hsu, C.; Xiong, D.; Wu, W.; Yu, Y.; Jia, H.; Wang, Y.; Zeng, Y.; Ji, M. *et al.* RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microb. Biotechnol.* (2020). DOI: 10.1111/1751-7915.13586

52. Yuan, X.; Wang, Y.; Meng, K.; Zhang, Y.; Xu, H.; Ai, W. LAMP real-time turbidity detection for fowl adenovirus. *BMC Vet. Res.* **15**, 256 (2019). DOI: 10.1186/s12917-019-2015-5

53. Zheney, M.; Kaziyev, Z.; Kassenova, G.; Zhao, L.; Liu, W.; Liang, L.; Li, G. Real-time fluorescence loop-mediated isothermal amplification assay for direct detection of egg drop syndrome virus. *BMC Vet. Res.* **14**, 49 (2018). DOI: 10.1186/s12917-018-1364-9

54. Jiang, M.; Pan, W.; Aratehfar, A.; Fang, W.; Ling, L.; Fang, H.; Daneshnia, F.; Yu, J.; Liao, W.; Pei, H. *et al.* Development and validation of a rapid single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system potentially to be used for reliable and high-throughput screening of COVID-19. *medRxiv* (2020). DOI: 10.1101/2020.03.15.20036376

55. El-Tholoth, M.; Bau, H. H.; Song, J. A single and two-stage, closed-tube, molecular test for the 2019 Novel Coronavirus (COVID-19) at home, clinic, and points of entry. (2020). DOI: 10.26434/chemrxiv.11860137.v1

56. Zhang, F.; Abudayyeh, O. O.; Jonathan, S. G. *A protocol for detection of COVID-19 using CRISPR diagnostics*. (2020). https://broad.io/sherlockprotocol.

57. Broughton, J. P.; Deng, X.; Yu, G.; Fasching, C. L.; Singh, J.; Streithorst, J.; Granados, A.; Sotomayor-Gonzalex, A.; Zorn, K.; Gopez, A. *et al.* Rapid detection of 2019 novel coronavirus SARS-CoV-2 using a CRISPR-based DETECTR lateral flow assay. *MedRxiv* (2020). DOI: 10.1101/2020.03.06.20032334

58. Kellner, M. J.; Koob, J. G.; Gootenberg, J. S.; Abudayyeh, O. O.; Zhang, F. SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nat. Protoc.* **14**, 2986–3012 (2019). DOI: 10.1038/s41596-019-0210-2

59. Basu, A.; Zinger, T.; Inglima, K.; Woo, K.; Atie, O.; Yurasits, L.; See, B.; Rosenfeld, M. E. A. Performance of the rapid Nucleic Acid Amplification by Abbott ID NOW COVID-19 in nasopharyngeal swabs transported in viral media and dry nasal swabs, in a New York City academic institution. *bioRxiv* (2020). DOI: 10.1101/2020.05.11.089896

60. Loeffelholz, M. J.; Alland, D.; Butler-Wu, S. M.; Pandey, U.; Perno, C. F.; Nava, A.; Carroll, K. C.; Mostafa, H.; Davies, E.; McEwan, A.; Rakeman, J. L. *et al.* Multicenter Evaluation of the Cepheid Xpert Xpress SARS-CoV-2 Test. *J. Clin. Microbiol.* (2020). DOI: 10.1128/JCM.00926-20

61. Visseaux, B.; Hingrat, Q. L.; Collin, G.; Bouzid, D.; Lebourgeois, S.; Pluart, D. L.; Deconinck, L.; Lescure, F-X.; Leucet, J-C.; Bouadma, D.*et al.* Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 Panel, the first rapid multiplex PCR commercial assay for SARS-CoV-2 detection. *J. Clin. Microbiol.* (2020). DOI: 10.1128/JCM.00630-20

62. Pachetti, M.; Marini, B.; Benedetti, F.; Giudici, F.; Mauro, E.; Storici, P.; Masciovecchio, C.; Angeletti, S.; Ciccozzi, M.; Gallo, R. C. *et al.* Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *J. Transl. Med.* **18**, 1–9 (2020). DOI: 10.1186/s12967-020-02344-6

63. Liu, D.; Daubendiek, S. L.; Zillman, M. A.; Ryan, K; Kool, E. T. Rolling circle DNA synthesis: small circular oligonucleotides as efficient templates for DNA polymerases. *J. Am. Chem. Soc.* **118**, 1587–1594 (1996). DOI: 10.1021/ja952786k

64. Wang, B.; Potter, S. J.; Lin, Y.; Cunningham, A. L.; Dwyer, D. E.; Su, Y.; Ma, X.; Hou, Y.; Saksena, N. K. Rapid and sensitive detection of severe acute respiratory syndrome coronavirus by rolling circle amplification. *J. Clin. Microbiol.* **43**, 2339–2344 (2005). DOI: 10.1128/JCM.43.5.2339-2344.2005

65. Zhao, W.; Ali, M. M.; Brook, M. A.; Li, Y. Rolling circle amplification: applications in nanotechnology and biodetection with functional nucleic acids. *Angew. Chem. Int. Ed.* **47**, 6330–6337 (2008). DOI: 10.1002/anie.200705982.

66. Koczula, K. M.; Gallotta, A. Lateral flow assays. *Essays Biochem.* **60**, 111–120 (2016). DOI: 10.1042/EBC20150012

67. Zhang, D.; Broyles D.; Hunt, E. A.; Dikici, E.; Daunert, S.; Deo, S. K. A paper-based platform for detection of viral RNA. *Analyst* **142**, 815–823 (2017). DOI: 10.1039/c6an02452a

68. Reboud, J.; Xu, G.; Garrett, A.; Adriko, M.; Yang, Z.; Tukahebwa, E. M.; Rowell, C.; Cooper, J. M. Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. *Proc. Natl. Acad. Sci.* **116**, 4834–4842 (2019). DOI: 10.1073/pnas.1812296116

69. Byers, K. M.; Bird, A. R.; Cho, H. D.; Linnes, J. C. Fully Dried Two-Dimensional Paper Network for Enzymatically Enhanced Detection of Nucleic Acid Amplicons. *ACS Omega* **5**, 4673–4681 (2020). DOI: 10.1021/acsomega.0c00115

70. Tanaka, R.; Yuhi, T.; Nagatani, N.; Endo, T.; Kerman, K.; Takamura, Y.; Tamiya, E. A novel enhancement assay for immunochromatographic test strips using gold nanoparticles. *Anal. Bioanal. Chem.* **385**, 1414–1420 (2006). DOI: 10.1007/s00216-006-0549-4

71. Giri, B. A perspective on the sensitivity of paper-analytical devices for bioanalysis. *J. Appl. Bionalysis* **2**, 6 (2016). DOI: 10.17145/jab.16.002

72. Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas 3rd, S. W.; Sindi, H.; Whitesides, G. M. Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem.* **80**, 3699–3707 (2008). DOI: DOI: 10.1021/ac800112r

73. Priye, A.; Bird, S. W.; Light, Y. K.; Ball, C. S.; Negrete, O. A.; Meagher, R. J. A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Sci. Rep.* **7**, 1–11 (2017). DOI: 10.1038/srep44778

74. Berg, B.; Cortazar, B.; Tseng, D.; Ozkan, H.; Feng, S.; Wei, Q.; Chan, R. Y.; Burbano, J.; Farooqui, Q.; Lewinski, M. *et al.* Cellphone-based hand-held microplate reader for point-of-care testing of enzyme-linked immunosorbent assays. *ACS Nano* **9**, 7857–7866 (2015). DOI: 10.1021/acsnano.5b03203

75. Laksanasopin, T.; Guo, T. W.; Nayak, S.; Sridhara, A. A.; Xie, S.; Olowookere, O. O.; Cadinu, P.; Meng, F.; Chee, N. H.; Kim, J. *et al.* A smartphone dongle for diagnosis of infectious diseases at the point of care. *Sci. Transl. Med.* **7** (2015). DOI: 10.1126/scitranslmed.aaa0056

76. Koirala, D.; Yu, Z.; Dhakal, S.; Mao, H. Detection of Single Nucleotide Polymorphism Using Tension-Dependent Stochastic Behavior of a Single-Molecule Template. *J. Am. Chem. Soc.* **133**, 9988–9991 (2011). DOI: 10.1021/ja201976r

77. Koirala, D.; Shrestha, P.; Emura, T.; Hidaka, K.; Mandal, S.; Endo, M.; Sugiyama, H.; Mao, H. Single-Molecule Mechanochemical Sensing Using DNA Origami Nanostructures. *Angew. Chem. Int. Ed.* **53**, 8137–8141 (2014). DOI: 10.1002/anie.201404043

78. Kaur, A.; Sapkota, K.; Dhakal, S. Multiplexed Nucleic Acid Sensing with Single-Molecule FRET. *ACS Sens.* **4**, 623–633 (2019). DOI: 10.1021/acssensors.8b01373

79. Rissin, D. M.; Kan, C. W.; Campbell, T. G.; Howes, S. C.; Fournier, D. R.; Song, L.; Piech, T.; Patel, P. P.; Chang, L.; Rivnak, A. J. *et al.* Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat. Biotechnol.* **28**, 595 (2010). DOI: 10.1038/nbt.1641

80. Greenwood, C.; Ruff, D.; Kirvell, S.; Johnson, G.; Dhillon, H. S.; Bustin, S. A. Proximity assays for sensitive quantification of proteins. *Biomol. Detect. Quantif.* **4**, 10–16 (2015). DOI: 10.1016/j.bdq.2015.04.002

81. Metzker, M. L. Sequencing technologies—the next generation. *Nat. Rev. Genet.* **11**, 31–46 (2010). DOI: 10.1038/nrg2626

82. Khan, M. J.; Trabuco, A. C.; Alfonso, H. L.; Figueiredo, M. L.; Batista, W. C.; Badra, S. J.; Figueiredo, L. T.; Lavrador, M. A.; Aquino, V. J. DNA microarray platform for detection and surveillance of viruses transmitted by small mammals and arthropods. *PLoS Negl. Trop. Dis.* **10**, (2016). DOI: 10.1371/journal.pntd.0005017

83. Shen, Z.; Xiao, Y.; kang, L.; Ma, W.; Shi, L.; Zhang, L.; Zhou, Z.; Yang, J.; Zhong, J.; Yang, D. *et al.* Genomic Diversity of Severe Acute Respiratory Syndrome–Coronavirus 2 in Patients With Coronavirus Disease 2019. *Clin Infect Dis* (2020). DOI: 10.1093/cid/ciaa203

84. Long, W.; Xiao, H.; Gu, X.; Zhang, Q.; Yang, H.; Zhao, G.; Liu, J. A universal microarray for detection of SARS coronavirus. *J. Virol. Methods* **121**, 57–63 (2004). DOI: 10.1016/j.jviromet.2004.06.016

85. Nouvellet, P.; Garske, T.; Mills, H. L.; Nedjati-Gilani, G.; Hinsley, W.; Blake, I. M.; Kerkhove, M. D. V.; Cori, A.; Dorigatti, I.; Jombart, T. *et al.* The role of rapid diagnostics in managing Ebola epidemics. *Nature* **528**, S109 (2015). DOI: 10.1038/nature16041

86. Fegan, D. F. Evaluation of diagnostic tests: the epidemiological approach. (Retrieved on June 3, 2020) http://www.fao.org/3/X4946E/x4946e0b.htm.

87. Lalkhen, A. G.; McCluskey, A. Clinical tests: sensitivity and specificity. *Contin. Educ. Anaesth. Crit. Care Pain* **8**, 221–223 (2008). DOI: 10.1093/bjaceaccp/mkn041

88. McNamara, L. A. & Martin, S. W. 1 - Principles of Epidemiology and Public Health. in Principles and Practice of Pediatric Infectious Diseases (Fifth Edition). *Elsevier.* (2018). DOI:10.1016/B978-0-323-40181-4.00001-3.

89. Udugama, B.; Kadhiresan, P.; Kozlowski, H. N.; Malekjahani, A.; Osborne, M.; Li, V. Y. C.; Chen, H.; Mubareka, S.; Gubbay, J. B.; Chan, W. C. W. Diagnosing COVID-19: The Disease and Tools for Detection. *ACS Nano* (2020). DOI: 10.1021/acsnano.0c02624

90. Dietz, K. The estimation of the basic reproduction number for infectious diseases. *Stat. Methods Med. Res.* **2**, 23–41 (1993). DOI: 10.1177/096228029300200103

91. Lipsitch, M.; Moxon, E. R. Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* **5**, 31–37 (1997). DOI: 10.1016/S0966-842X(97)81772-6

92. Guerra, F. M.; Bolotin, S.; Lim, G.; Heffernan, J.; Deeks, S. L.; Li, Y.; Crowcroft, N. S. The basic reproduction number (R0) of measles: a systematic review. *Lancet Infect. Dis.* **17**, e420–e428 (2017). DOI: 10.1016/S1473-3099(17)30307-9

93. Kretzschmar, M.; Teunis, P. F.; Pebody, R. G. Incidence and reproduction numbers of pertussis: estimates from serological and

social contact data in five European countries. *PLoS Med.* **7**, (2010). DOI: 10.1371/journal.pmed.1000291

94. Australian Government Department of Health. Mumps Laboratory Case Definition (LCD). (2015) https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-phlncd-mumps.htm

95. Ferguson, N. M.; Cummings, D. A. T.; Fraser, C.; Cajka, J. C.; Cooley, P. C.; Burke, D. S. Strategies for mitigating an influenza pandemic. *Nature* **442**, 448–452 (2006). DOI: 10.1038/nature04795

96. Fraser, C.; Donnelly, C. A.; Cauchemez, S.; Hanage, W. P.; Van Kerkhove, M. D.; Hollingsworth, T. D.; Griffin, J.; Baggaley, R. F.; Jenkins, H. E.; Lyons, E. J. *et al.* Pandemic potential of a strain of influenza A (H1N1): early findings. *science* **324**, 1557–1561 (2009). DOI: 10.1126/science.1176062

97. Kucharski, A. J.; Althaus, C. The role of superspreading in Middle East respiratory syndrome coronavirus (MERS-CoV)

transmission. *Euro Surveill.* **20** (2015). DOI: 10.2807/1560-7917.es2015.20.25.21167

98. Khan, A.; Naveed, M.; Dur-e-Ahmad, M.; Imran, M. Estimating the basic reproductive ratio for the Ebola outbreak in Liberia and Sierra Leone. *Infect. Dis. Poverty* **4**, 13 (2015). DOI: 10.1186/s40249-015-0043-3

99. Gani, R.; Leach, S. Transmission potential of smallpox in contemporary populations. *Nature* **414**, 748–751 (2001). DOI: 10.1038/414748a

100. Chen, J. Pathogenicity and transmissibility of 2019-nCoV—a quick overview and comparison with other emerging viruses. *Microbes Infect.* (2020). DOI: 10.1016/j.micinf.2020.01.004

101. Roser, M.; Ritchie, H.; Ortiz-Ospina, E. Coronavirus Disease (COVID-19) – Statistics and Research. *Our World Data* (2020). https://ourworldindata.org/coronavirus
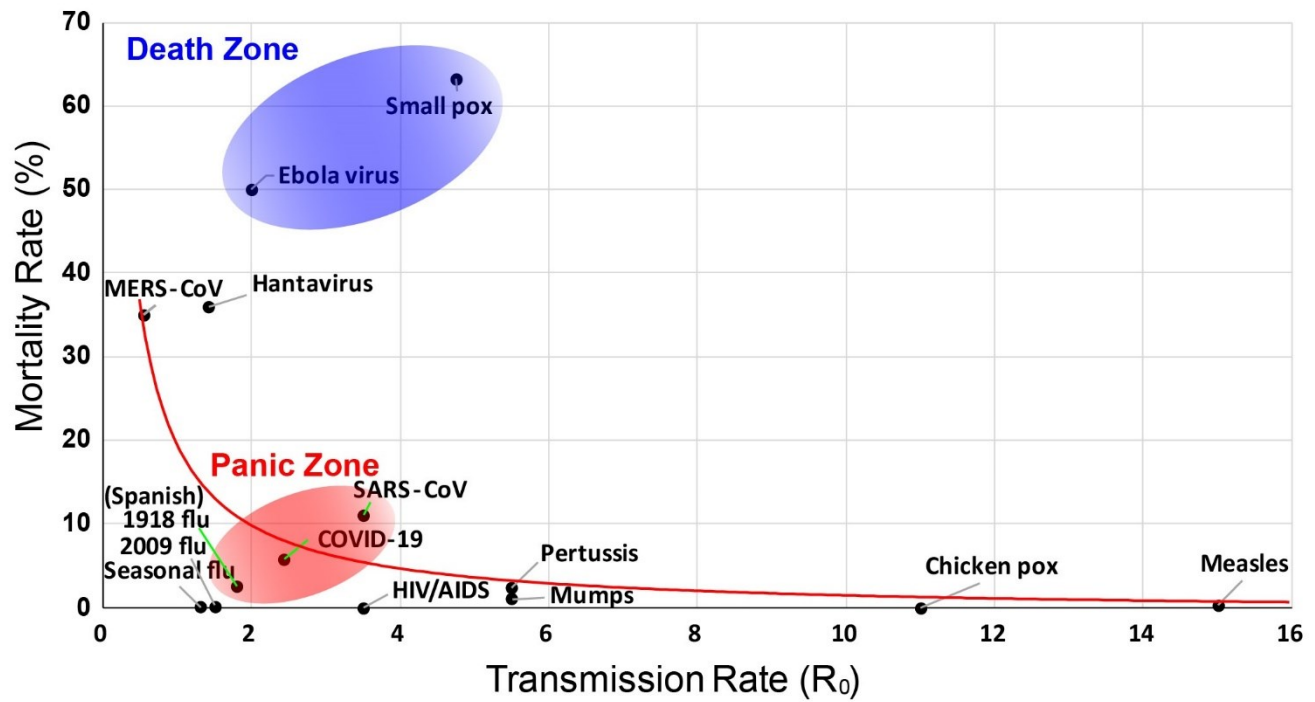
FIGURE 1. Viruses with high transmission rates (R_0) are less fatal. R_0 is the reproduction rate of a virus, which measures its transmissibility[77]. Solid curve represents an inverse fitting between the mortality rate and the R_0, which has been proposed as the trade-off principle between the virulence and transmissibility of virus[91]. 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[92–100].
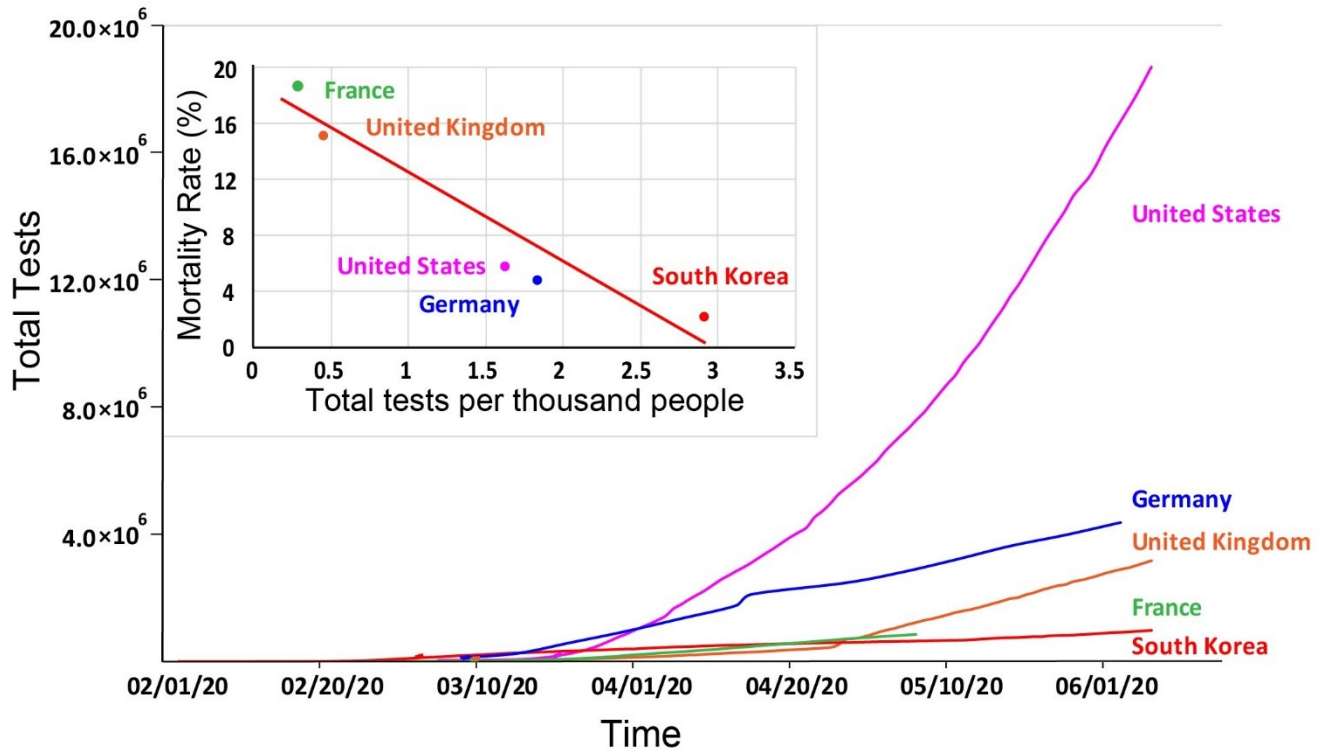
FIGURE 2. Critical importance of the early detection in the COVID-19 outbreak. COVID-19 daily tests are shown for 5 countries with similar medical resources and age distributions. Inset shows the mortality rates (percentage of the death cases among all confirmed COVID-19 cases) as of 06/07/2020 vs the number of the early detections per thousand population performed during 03/04/2020 - 03/26/2020. The early detection data for each country[101] 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.
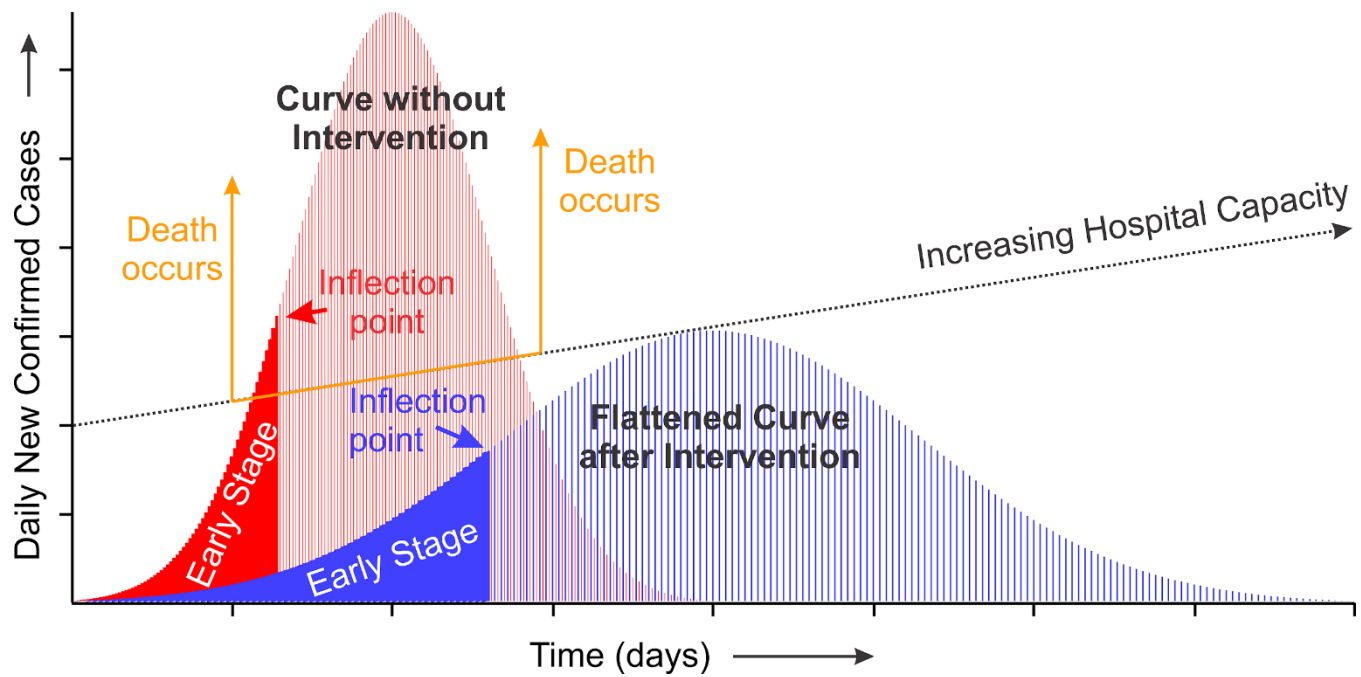
FIGURE 3. Intervention of the COVID-19 outbreak. The intervention at an early stage (before the inflection point, which is the point where the half width of a Gaussian peak is equivalent to the sigma of the Gaussian) of a viral breakout is the key to slow down the transmission of the virus. It not only 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.
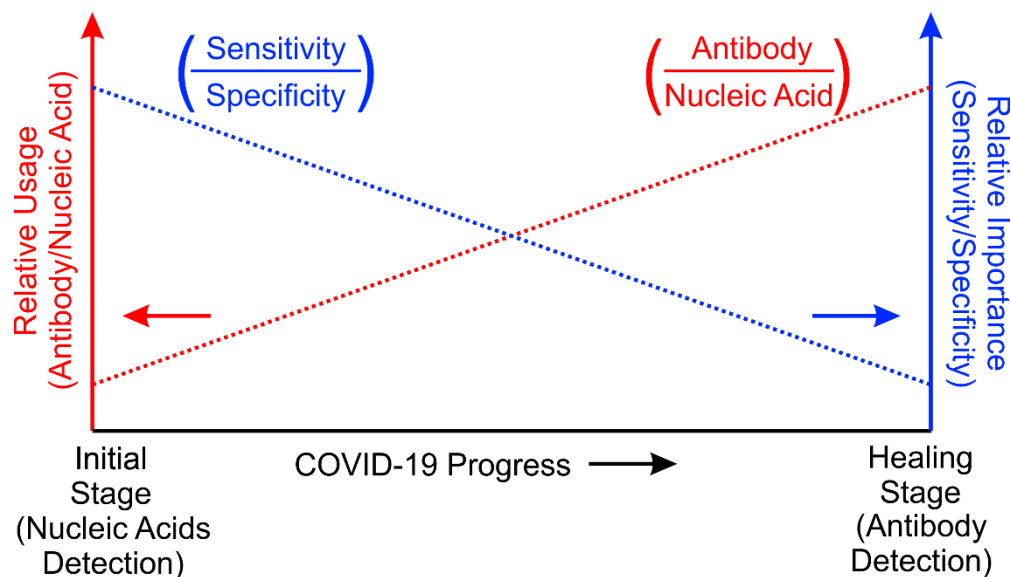
FIGURE 4. Schematic diagram of the relative usage of nucleic acid vs antibody detection methods (left y axis) and the relative importance of sensitivity vs specificity (right y axis) in the detection of SARS-CoV-2 virus during the COVID-19 pandemic. At the initial breakout stage, nucleic acid-based detection 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.

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	-
06/18/2020*	3B Blackbio Biotech India Ltd., a subsidiary of Kilpest India Ltd.	RT-PCR	E, RdRP, N, RNaseP	10 cp/µL	-
03/2020#	3D Medicines	RT-PCR	-	-	-
€	A*Star Tan Tock Seng Hospital of Singapore	RT-PCR	-	-	-
3/27/2020*	Abbott Diagnostics Scarborough, Inc.	RT-LAMP	RdRP	125 GE/mL	5-13 min
3/18/2020*	Abbott Molecular	RT-PCR	RdRP, N	100-200 cp/mL	-
05/11/2020*	Abbott Molecular Inc.	RT-PCR	RdRp, N	100 cp/mL	-
06/29/2020*	Acupath Laboratories, Inc.	RT-PCR	ORF1ab, N, S	25 cp/µL	-
04/22/2020*	Altona Diagnostics GmbH	RT-PCR	N, S	0.1 PFU/mL	-
02/2020#	Anatolia Geneworks	RT-PCR	-	-	-
06/15/2020*	Applied Biocode, Inc.	RT-PCR	N	1.72 x10-2 TCID50/mL	-
05/13/2020*	Applied DNA Sciences, Inc.	RT-PCR	RNaseP	5 cp/rxn	-
**	ARUP Laboratories	RT-PCR	-	-	-
05/15/2020*	Assurance Scientific Laboratories	RT-PCR	N1, N2, RNaseP	37 cp/rxn	-
4/10/2020*	Atila BioSystems, Inc	RT-PCR	N, ORF1ab	4 cp/µL	-
03/2020#	AUSDiagnostics	RT-PCR	-	-	-
3/25/2020*	Avellino Lab USA, Inc.	RT-PCR	RNase P (RP)	55 cp/µL	1-2 days
4/8/2020*	Becton, Dickinson & Company	RT-PCR	N, RP	40 GE/mL	-
-	Beijing Applied	RT-PCR	ORF1ab, N, E	1000 cp/mL	90 min
3/26/2020*, 3/2/2020#, 1/2020$	BGI Genomics Co. Ltd.	RT-PCR	ORF1ab	100 cp/mL	-
-	BGI Wuhan Biotech Co., Ltd	RT-PCR	ORF1ab	100 cp/mL	90 min
05/21/2020*	BioCore Co., Ltd.	RT-PCR	N, RdRp	500 cp/mL	-
3/23/2020*	BioFire Defense, LLC	RT-PCR	ORF1ab, ORF8	330 cp/mL	50 min
05/01/2020*	BioFire Diagnostics, LLC	Multiplex RT-PCR	-	-	-
05/06/2020*	BioMérieux SA	RT-PCR	N, RdRp, E	300 GE/mL	-
-	Bioneer	RT-PCR	-	-	-
05/01/2020*	Bio-Rad Laboratories, Inc	Endpoint RT-PCR	N1, N2	625 cp/mL	-
**	BioReference Laboratories	RT-PCR	-	-	-
2/4/2020*	Centers for Disease Control and Prevention's (CDC)	RT-PCR	N1, N2. RP	4-10 cp/µL	-
3/20/2020*	Cepheid	RT-PCR	N2, E	250 cp/mL	45 min
03/2020	CerTest BioTec	RT-PCR	-	-	-
06/09/2020*	ChromaCode Inc.	RT-PCR	N1, N2, RNase P	500 cp/mL	-
4/3/2020*	Co-Diagnostics, Inc	RT-PCR	RdRP	600 cp/spl	-
03/2020#	Credo Diagnostics Biomedical	RT-PCR	-	-	-
06/10/2020*	Cue Health Inc.	Isothermal amplification	N	20 GC/sample	-
-	Daan Gene Co., Ltd., Sun Yat-sen University	RT-PCR	ORF1ab, N	500 cp/mL	110 min
05/22/2020*	dba SpectronRX	RT-PCR	E, N	5 cp/rxn	-

Date	Company	Method	Gene Targets	LoD	Time
4/8/2020*	DiaCarta, Inc	RT-PCR	E, N, ORF1ab	100 cp/mL	-
****	Diagnostic Solutions Laboratory	RT-PCR	-	-	-
06/25/2020*	Diagnostic Solutions Laboratory, LLC	RT-PCR	N1, N3, S	10 cp/swab	-
3/19/2020*	DiaSorin Molecular LLC	RT-PCR	ORF1ab, S	500 cp/mL	1-1.5 h
-	Diatherix Eurofins	RT-PCR	-	-	-
06/08/2020*	Euroimmun US, Inc.	RT-PCR	N, ORF1ab	150 cp/mL	-
05/15/2020*	Flugent Therapeutics, LLC	RT-PCR	N1, N2, RNaseP	5 cp/mL	-
04/17/2020*	Fosun Pharma USA Inc.	RT-PCR	ORF1ab, N, E	300 cp/mL	-
**	Fulgent Genetics/MedScan laboratory	Sequencing	-	-	-
06/23/2020*	Gencurix, Inc.	RT-PCR	ORF1ab, E	6000 GE/mL	-
05/14/2020*	GeneMatric, Inc.	RT-PCR	RdRp, N	50 cp/rxn	-
03/2020#	Genetic Signatures	RT-PCR	-	-	-
06/05/2020*	Genetron Health (Beijing) Co., Ltd.	RT-PCR	ORF1ab, N, RNaseP	1000 cp/mL	-
3/19/2020*	GenMark Diagnostics, Inc.	RT-PCR, electrowetting and sensing	-	10^5 cp/mL	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, N3	4.8 cp/μL	-
06/23/2020*	HealthQuest Esoterics	RT-PCR	N, S, ORF1ab, MS2	20 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} TCID50/mL	-
06/09/2020*	Illumina, Inc.	Next Gen Sequencing	-	1000 cp/mL	-
4/7/2020*	InBios International, Inc	RT-PCR	E, N, ORF1ab	12.5 GE/rxn	-
06/26/2020*	Inform Diagnostics, Inc.	RT-PCR	N1, N2	20 cp/μL	-
*	Integrated DNA technologies/Danaher	RT-PCR	-	-	-
4/1/2020*	Ipsum Diagnostics, LLC	RT-PCR	N, RP	8.5 cp/μL	-
€	JN Medsys	RT-PCR	-	-	-
#, Ψ	Kogene Biotech	RT-PCR	-	-	-
4/16/2020*	KorvaLabs, Inc	RT-PCR	N1, N2, RP	200 cp/rxn	-
04/29/2020*	LabGenomics Co., Ltd.	RT-PCR	RdRp, E	20 GE/mL	-
3/16/2020*	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	-
4/3/2020*	Luminex Corporation	RT-PCR	ORF1ab, N	75 GE/uL	-
3/27/2020*	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, Inc	RT-PCR	Nsp2, N	150 cp/mL	
3/20/2020*, 2/2020#, 3/26/2020λ	Novacyt/Primerdesign	RT-PCR	-	-	-
06/17/2020*	Omnipathology Solutions Medical Corporation	RT-PCR	N1, N2	1.23 cp/μL	-

Date	Manufacturer	Method	Gene Target	LoD	Time
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	-
06/25/2020*	PlexBio Co., Ltd.	RT-PCR	RdRP, E, N	140 cp/mL	-
06/25/2020*	PreciGenome LLC	RT-PCR	ORF1ab, N, RNaseP	571.4 cp/mL	-
3/20/2020*	Primerdesign Ltd.	RT-PCR	-	0.33 cp/µL	-
***	Promedical	Lateral Flow Immunoassay	-	-	-
06/30/2020*	Psomagen, Inc.	RT-PCR	N1, N2, hRP	1 cp/µL	-
3/30/2020*	QIAGEN GmbH	RT-PCR	-	500 cp/mL	-
3/17/2020*	Quest Diagnostics Infectious Disease, Inc.	RT-PCR	N1 and N3	136 cp/mL	-
05/18/2020*	Quidel Corporation	RT-PCR	Pp1ab	1.28×10^4 Genome eq/mL	75 min
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
06/12/2020*	RTA Laboratories Biological Products Pharmaceutical and Machinery Industry	RT-PCR	E, RdRP, RNaseP	100 cp/mL	-
05/04/2020*	Sansure BioTech Inc.	RT-PCR	ORF1ab, N	200 cp/mL	90 min
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*	See gene, 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	-
05/21/2020*	SolGent Co., Ltd.	RT-PCR	N, ORF1	200 cp/mL	-
03/2020#	Systaaq Diagnostic Products	RT-PCR	-	-	-
06/10/2020*	TBG Biotechnology Corp.	RT-PCR	RdRP, E, N, RNase P	10 cp/µL	-
06/17/2020*	The Ohio State University Wexner Medical Center	RT-PCR	N1, N2, RNaseP	0.25 cp/µL	-
3/13/2020*	Thermo Fisher Scientific, Inc.	RT-PCR	S, N	10 GE/rxn	4 h
03/2020#	TIB MolBiol Synthesalabor	RT-PCR	E	-	-
06/10/2020*	Tide Laboratories, LLC	RT-PCR	N	20 cp/µL	-
06/30/2020*	TNS Co., Ltd (Bio TNS)	RT-PCR	N, RdRP	2.5 cp/µL	-
04/20/2020*	Trax Management Services Inc.	RT-PCR	RNaseP	50 cp/mL	-
06/23/2020*	University of Alabama at Birmingham Fungal Reference Lab	RT-PCR	N1, RNaseP	125 cp/mL	-
06/24/2020*	University of Texas MD Anderson Cancer Center, Molecular Diagnostics Laboratory	RT-PCR	N1, N2	5 cp/µL	-
-	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

Table 2: Kits based on antibodies detection

Authorization	Manufacturer	Mechanism	Target	LOD	Time
04/26/2020*	Abbott Laboratories, Inc.	Chemiluminescent micro-particle immunoassay	IgG	-	-
***	Assure Tech	Immunoassay	IgG IgM	-	-
***	Autobio Diagnostics	Immunoassay	-	-	-
04/24/2020*	Autobio Diagnostics Co. Ltd.	Lateral Flow Immunoassay	IgG IgM	-	50 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	-	-	-
***	Beroni Group	Immunoassay	IgG IgM	-	-
***	BioMedomics	Immunoassay	IgG IgM	-	-
-	BiOSCiENCE	Immunoassay	IgM IgG	-	30 min
***	BTNX	Immunoassay	IgG IgM	-	-
4/1/2020*	Cellex	Immunoassay	IgG IgM	-	-
***	ChemBio Diagnostic System	Immunoassay	IgG IgM	-	-
4/14/2020*	Chembio Diagnostic System, Inc	Immunoassay	IgG IgM	-	-
***	Core Technology	Immunoassay	IgG IgM	-	-
04/24/2020*	DiaSorin Inc.	Chemiluminescent Immunoassay	IgG	-	-
***	Diazyme Laboratories	Immunoassay	IgG IgM	-	-
***	Eachy Biopharmaceuticals	Immunoassay	IgG IgM	-	-
-	Eagle Bioscience	Immunoassay	IgG, IgM	-	-
05/04/2020*	EUROIMMUN US Inc.	ELISA	IgG	-	2.5 h
-	Guangdong Hecin	Immunoassay	IgM	-	-
***	Guangzhou Wondfo	Immunoassay	-	-	15 min
***	Hangzhou AllTest Biotech	Immunoassay	IgG IgM	-	-
***	Hangzhou Biotest Biotech	Immunoassay	IgG IgM	-	-
06/04/2020*	Hangzhou Biotest Biotech Co., Ltd.	Lateral Flow Immunoassay	IgG IgM	-	30 min
***	Hangzhou Clongene Biotech	Immunoassay	IgG IgM	-	-
***	Hangzhou Testsealabs Biotechnology	Immunoassay	IgG IgM	-	-
***	Healgen Scientific	Immunoassay	IgG IgM	-	-
05/29/2020*	Healgen Scientific LLC	Lateral Flow Immunoassay	IgG IgM	-	10 min
-	INNOVITA (Tangshan)	Immunoassay	IgG IgM	-	15 min
***	Jiangsu Macro & Micro-Test Med-Tech	Colloidal gold	IgG IgM	-	-
***	Lifeassay Diagnostics	Immunoassay	IgG IgM	-	-
***	Medical Systems Biotechnology	Immunoassay	IgG IgM	-	-
4/15/2020*	Mount Sinai Laboratory	Immunoassay	IgG	NA	-
***	Nanjing Liming Bio-Products	Immunoassay	IgG IgM	-	-
-	Nanjing Vazyme	Immunoassay	IgM, IgG	-	10 min
***	NanoResearch	Immunoassay	IgG IgM	-	-
***	Nantong Diagnos Biotechnology	Colloidal gold	-	-	-
***	Nirmidas Biotech	Immunoassay	IgG IgM	-	-
4/14/2020*	Ortho Clinical Diagnostics, Inc	Lateral Flow Immunoassay	IgG IgM	-	10-15 min
***	PCL	Immunoassay	IgG IgM	-	-

***	PharmaTech	Immunoassay	IgG IgM	-	-
05/08/2020*	Quidel Corporation	Lateral Flow Immunoassay	Nucleocapsid protein	-	15 min
06/02/2020*	Roche Diagnostics	Immunoassay	IL-6	-	18 min
05/02/2020*	Roche Diagnostics	Immunoassay	-	-	18 min
***	SD Biosensor	Immunoassay	IgG IgM	-	-
***	Shenzhen Landwind Medical	Immunoassay	IgG IgM	-	-
05/29/2020*	Siemens Healthcare Diagnostics Inc.	Chemiluminescent Immunoassay	Total Antibodies	-	10 min
02/2020#	Snibe Diagnostics	Immunoassay	IgG IgM	-	-
***	Telepoint Medical Services	Immunoassay	IgG IgM	-	-
***	Tianjin Beroni Biotechnology	Immunoassay	IgG IgM	-	-
06/04/2020*	Vibrant America Clinical Labs	Chemiluminescence immunoassay	IgG IgM	-	-
04/30/2020*	Wadsworth Center, New York, State Department of health	Microsphere Immunoassay	Total Antibodies	-	-
-	Xiamen innoDx Bio-tech	Immunoassay	IgG IgM	-	-
***	Zuhai Livzon Diagnostics	Colloidal gold	IgG IgM	-	15 min

Table 3: Kits based on 'not identified' mechanism

Authorization	Manufacturer	Mechanism	Target	LOD	Time
-	Biological Technologies Co., LTD	-	-	-	-
-	(Chongqing) Bio-tech, Co., Ltd	-	-	-	-
-	Health Technology Co., Ltd	-	-	-	-
-	Bio-tech Co., Ltd	-	-	-	-
-	Medical Technology Co., Ltd	-	-	-	-
-	Biotechnologies (Hangzhou) Ltd	-	-	-	-
-	Biomedicine Co., Ltd.	-	-	-	-

*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 1-3:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

http://ph.china-embassy.org/eng/sgdt/t1760281.htm.

https://www.modernhealthcare.com/safety/coronavirus-test-tracker-commercially-available-covid-19-diagnostic-tests.

https://www.finddx.org/covid-19/pipeline/.

For TOC only