

# Detecting the Coronavirus (COVID-19)

Pravin Pokhrel, Changpeng Hu, and Hanbin Mao\*

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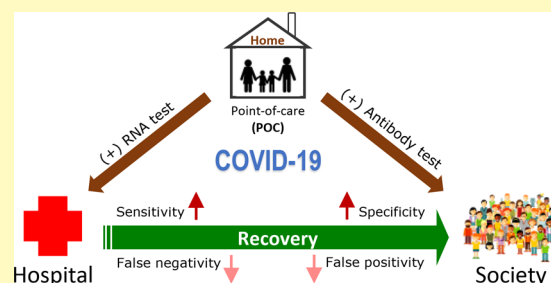
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**ABSTRACT:** The COVID-19 pandemic has created huge damage to society and brought panic around the world. Such panic can be ascribed to the seemingly deceptive features of COVID-19: Compared to other deadly viral outbreaks, it has medium transmission and mortality rates. As a result, the severity of the causative coronavirus, SARS-CoV-2, was deeply underestimated by 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 the current COVID-19 pandemic. As of July 2020, more than 100 kits for COVID-19 diagnosis on the market have been 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 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 importance at later stages of the outbreak. When society is set to reopen from the lockdown 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 society after their recovery from 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 the private sector 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 massive POCT) to contain Panic Zone viruses in the future.

**KEYWORDS:** diagnosis, detection kits, RT-PCR, immunoassay, false negative, false positive, sensitivity, specificity, point-of-care testing (POCT)



## 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 society. Loss of life struck the most, but the consequences in the aftermath were equally dreadful: the psychological well-being of survivors and the 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).

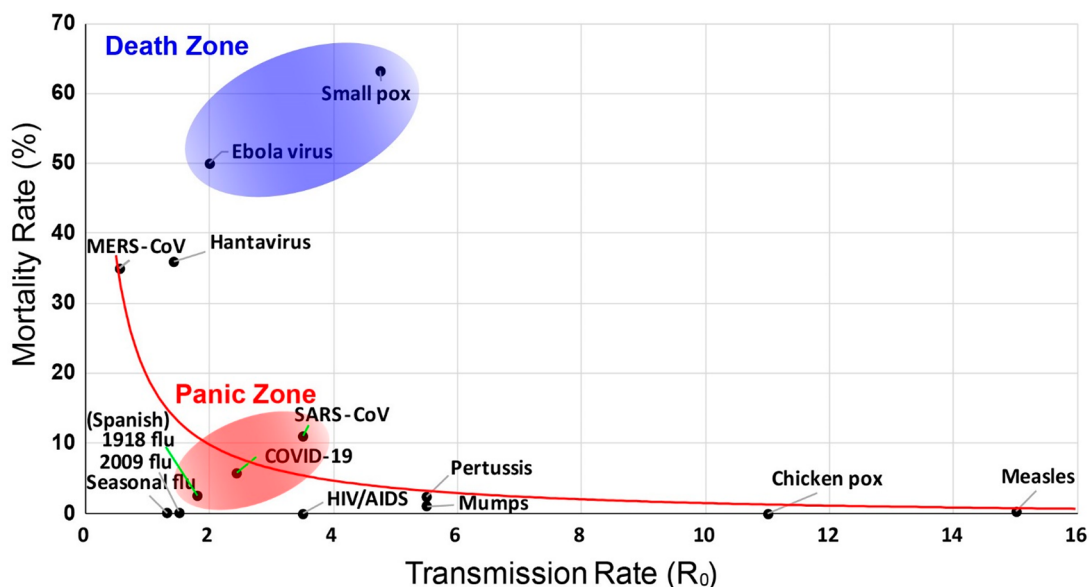
**The Panic Zone Viruses.** Compared to other viruses, SARS-CoV-2 has a medium reproduction rate ( $R_0 = 2.25^*$ ) and a medium mortality rate of 5.7%<sup>\*1</sup> (as of June 7, 2020, \*subject to change). Such mediocre characteristics give a rather deceiving impression of this virus. When the virus 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 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 healthcare systems in society. This eventually caused panic 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.<sup>2</sup> 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,

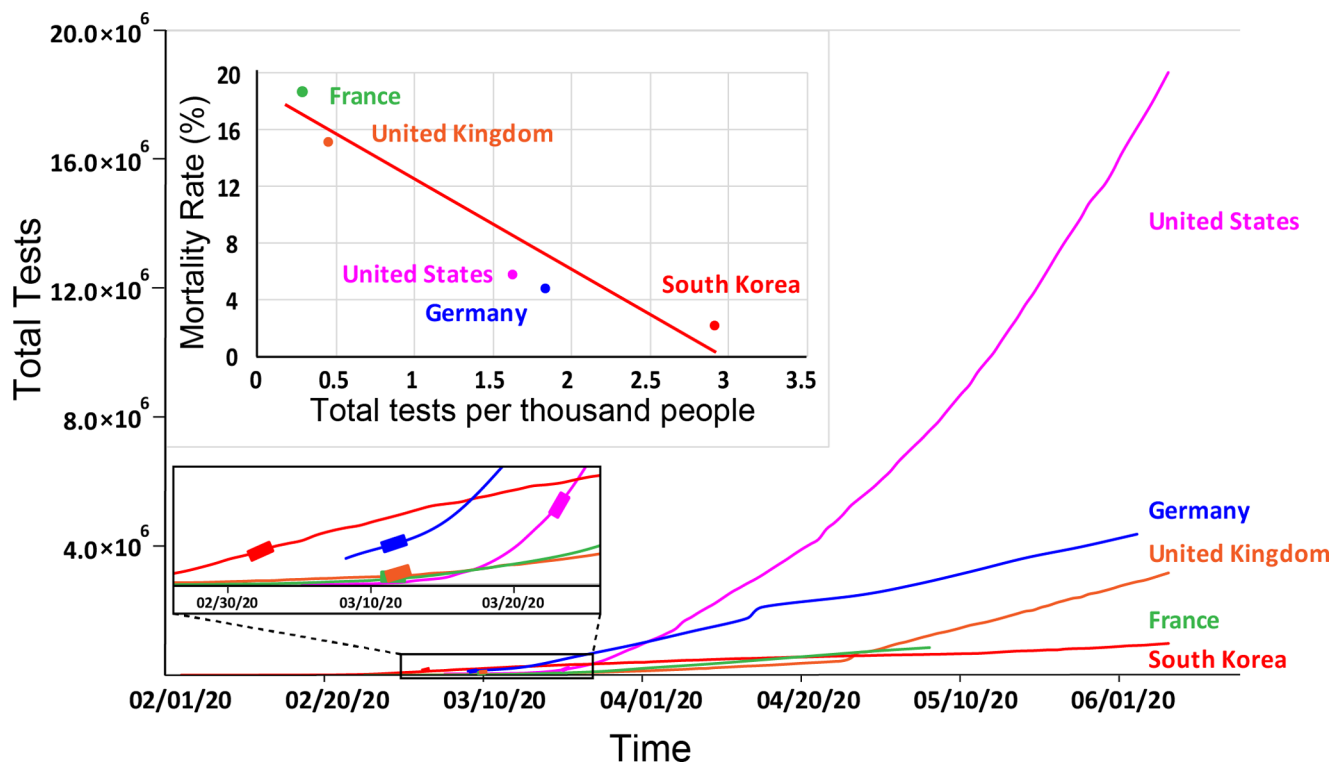
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**Figure 1.** Viruses with high transmission rates ( $R_0$ ) are less fatal.  $R_0$  is the reproduction rate of a virus, which measures its transmissibility.<sup>90</sup> Solid curve represents an inverse fitting between the mortality rate and the  $R_0$ , which has been proposed as the trade-off principle between the virulence and the transmissibility of the virus.<sup>91</sup> 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 refs 92–100.



**Figure 2.** Critical importance of 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 June 7, 2020 vs the number of early detections per thousand population performed during March 4–March 26, 2020. The early detection data for each country<sup>101</sup> 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.

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 wreak huge collateral damage due to their 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.<sup>3</sup> 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 community spread.<sup>3</sup> Although COVID-19 has lower transmissibility than SARS,<sup>4</sup> 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.

**Early Detection.** It is generally true that for a rapidly transmitted disease with no cure or vaccine available, the most effective way to curb its spread is early detection to isolate patients.<sup>5,6</sup> 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 testing on COVID-19, the lower the overall mortality rate 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 early testing to curb the spread of COVID-19.

In this review, we first describe the COVID-19 outbreak briefly. Given the importance of 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 was reported in Wuhan, China.<sup>7</sup> The causative virus of that disease was determined to be 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.<sup>8</sup> 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 research, the clinical characteristics for COVID-19 appear 1–14 days after infection, and most patients develop symptoms within 3–7 days.<sup>9</sup> The common symptoms include fever, coughing, and body weakness. A few patients develop nasal congestion, runny 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.<sup>9</sup> 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.<sup>10</sup> The elderly and patients with chronic underlying diseases had poor prognosis.<sup>11</sup>

**Epidemiology of COVID-19.** People are generally susceptible to 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 an object surface, which then enters the body via the mouth, nose, eyes, or other mucous membrane<sup>12</sup>). Study has shown a higher viral load in the nasal cavity than the throat, suggesting that nasal sampling is a more effective approach to detect the virus. There was no difference in the viral load between symptomatic and asymptomatic patients,<sup>13</sup> the latter of which can also transmit the disease.<sup>14</sup> Guan et al. reported that some patients tested positive for SARS-CoV-2 in stool and urine samples also.<sup>9</sup>

## ■ DIAGNOSIS OF 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.<sup>15–18</sup> These features demonstrate a highly organized pattern of pneumonia.<sup>16</sup> Unlike these features, nodules, cystic changes, bronchiectasis, pleural diffusion, and lymphadenopathy are less common.<sup>18</sup>

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.<sup>19</sup> This is because features of the chest imaging from COVID-19 patients may overlap with other infections caused by influenza, H1N1, or SARS-CoV.<sup>20,21</sup>

However, studies on the sensitivity of CT imaging over RT-PCR (Reverse Transcription - Polymerase Chain Reaction, which is considered the reference standard for laboratory testing of SARS-CoV-2; see [Nucleic Acid Based Methods](#) section) showed that CT imaging is more sensitive and reliable in detecting SARS-CoV-2 infections during certain stages of the COVID-19. Fang et al. studied 51 patients with COVID-19 symptoms based on their clinical manifestations and epidemiological histories.<sup>22</sup> 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.<sup>23</sup> 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 that 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

Table 1. Kits Based on Nucleic Acid Detection<sup>a</sup>

Authorization	Manufacturer <sup>b</sup>	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 <sup>#</sup>	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 <sup>#</sup>	Anatolia Geneworks	RT-PCR	-	-	-
06/15/2020*	Applied Biocode, Inc.	RT-PCR	N	1.72 × 10 <sup>-2</sup> 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 <sup>#</sup>	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 <sup>#</sup> , 1/2020 <sup>S</sup>	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.	End point 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 <sup>#</sup>	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	-
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*	GeneMatic, Inc.	RT-PCR	RdRp, N	50 cP/rxn	-
03/2020 <sup>#</sup>	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 <sup>5</sup> cP/mL	2 h
03/2020 <sup>#</sup>	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	-

Table 1. continued

Authorization	Manufacturer <sup>b</sup>	Mechanism	Target	LOD	Time
05/14/2020*	Hologic, Inc.	Transcription Mediated Amplification	ORF1ab	0.01 TCID <sub>50</sub> /mL	-
3/16/2020*	Hologic, Inc.	RT-PCR	-	10 <sup>-2</sup> TCID <sub>50</sub> /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*	Ipsium 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 Laboratories	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 <sup>#</sup> , 3/26/2020 <sup>Δ</sup>	Novacyt/Primerdesign	RT-PCR	-	-	-
06/17/2020*	Omnipathology Solutions Medical Corporation	RT-PCR	N1, N2	1.23 cP/μL	-
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 <sup>#</sup>	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 <sup>4</sup> Genome eq/mL	75 min
03/2020\$	Rendu Biotechnology	RT-LAMP	-	-	-
04/29/2020*	Rheonix, Inc.	End point RT-PCR	N1	625 GE/mL	-
3/12/2020*	Roche Molecular Systems, Inc.	RT-PCR	E	-	3 h
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 Biotech 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 <sup>#</sup>	Systaaq Diagnostic Products	RT-PCR	-	-	-
06/10/2020*	TBG Biotechnology Corp.	RT-PCR	RdRP, E, N, RNaseP	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

Table 1. continued

Authorization	Manufacturer <sup>b</sup>	Mechanism	Target	LOD	Time
03/2020 <sup>#</sup>	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 <sup>#</sup>	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

<sup>a</sup>\*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, €Singapore Health Sciences Authority, personal authorization for clinical use, λEUA India, ηKorea Centers for Disease Control and the Korea Food and Drug Administration, - Data Not Available. <sup>b</sup>References for kits: <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/>.

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 spread of 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.<sup>22</sup> 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 SARS-CoV-2 as the causative virus for this pandemic, the SARS-CoV-2 genome was quickly sequenced,<sup>24</sup> from which unique sequences have been identified for COVID-19 diagnosis. Reverse transcription-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.<sup>25</sup> 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<sup>26</sup> to quantify the target, whereas nested RT-PCR uses two sets of primers to avoid nonspecific PCR amplifications.<sup>27</sup>

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.<sup>28</sup> This assay showed a high dynamic range of 0.0002–20 TCID<sub>50</sub> (50% tissue culture infective dose) per reaction and 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.<sup>29</sup> 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.<sup>29</sup> This method further increased sensitivity to detect as few 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.<sup>29</sup> In France, two RdRP genes were used for initial screening followed by the confirmatory E gene testing.<sup>29</sup> In Japan, nested RT-PCR was used,<sup>29</sup> which significantly reduced nonspecific 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 a 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 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.<sup>30</sup> Indeed, the Chinese authority has adopted this approach to diagnose COVID-19 in Wuhan by combining RT-PCR with CT scans.<sup>23</sup> 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 for use in RT-PCR,<sup>31</sup> while Yam et al. concluded that testing more than one specimen could significantly maximize the sensitivity of the RT-PCR testing.<sup>32</sup> These findings suggest that it is rather important to apply nucleic acid based kits with optimized conditions to maximize their diagnostic potency. In particular, the finding of effective SARS-CoV-2 detection in the noninvasive saliva<sup>33</sup> provides a convenient way to develop affordable point-of-care testing kits that can be massively used by the general public (see *Ideal*

Table 2. Kits Based on Antibody Detection<sup>a</sup>

Authorization	Manufacturer <sup>b</sup>	Mechanism	Target	LOD	Time
04/26/2020*	Abbott Laboratories, Inc.	Chemiluminescent microparticle 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 <sup>#</sup>	Snibe Diagnostics	Immunoassay	IgG IgM	-	-
***	Telepoint Medical Services	Immunoassay	IgG IgM	-	-
***	Tianjin Beroni Biotechnology	Immunoassay	IgG IgM	-	-
06/04/2020*	Vibrant America Clinical Laboratories	Chemiluminescence immunoassay	IgG IgM	-	-
04/30/2020*	Wadsworth Center, New York, State Department of health	Microsphere Immunoassay	Total Antibodies	-	-
-	Xiamen innoDx Biotech	Immunoassay	IgG IgM	-	-
***	Zuhai Livzon Diagnostics	Colloidal gold	IgG IgM	-	15 min

<sup>a</sup>\*US EUA Authorized, \*\*\*US Notified FDA under section IV.D, #European Union Conformity Marked, - Data Not Available. <sup>b</sup>References for kits: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics>

Table 2. continued

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/>.

### Characteristics of Diagnostic Methods and Emerging Techniques to Detect SARS-CoV-2 sections).

Table 1 lists the nucleic acid based kits used for the diagnosis of COVID-19. The sensitivity of those kits ranges from 100 to 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.<sup>34</sup> In a study of post symptomatic patients, Amanat et al. detected high IgA and IgM immune responses.<sup>35</sup> 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%.<sup>36</sup> 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 capability to bind to SARS-CoV-2 antibodies.<sup>37</sup> In a comparative study, both ELISA and colloidal gold immunochromatographic kits showed equal sensitivity with 100% specificity for the SARS-CoV-2 detection.<sup>38</sup>

Several immunoassay kits are already on the 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,<sup>39</sup> potential cross reactivity (less specific),<sup>40</sup> and low sensitivity with respect to nucleic acid based methods. Nevertheless, immunoassays are faster<sup>41</sup> 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 stages of society at which people recovered from previous COVID-19 infections, and therefore immune to the virus, can safely re-engage with 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: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users.<sup>42</sup> 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. However, 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)<sup>43</sup> 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<sup>44</sup> 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<sup>45</sup> 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 (Diagnosis of COVID-19 section), 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 Nucleic Acid Based Methods section), it is time-consuming and requires a sophisticated laboratory facility and trained personnel to operate.<sup>46</sup> 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 °C.<sup>47</sup> 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 and 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.<sup>48–51</sup> As a result, the target sequence is amplified exponentially, which is detected by turbidimetry<sup>52</sup> or fluorescence<sup>53</sup>/colorimetry.<sup>48</sup>

As an example, RNA extraction and LAMP amplification have been performed in the same tube.<sup>51,54</sup> This method has LOD ranging 80–500 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.<sup>55</sup> 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's group<sup>56</sup> and Chiu's group<sup>57</sup> integrated the LAMP with the CRISPR-based SHERLOCK (see Table 1)<sup>58</sup> and CRISPR-Cas12 based methods, respectively, to detect the SARS-CoV-2 RNA with a detection limit as low as 10 copies/ $\mu$ L 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 is such an example. This method only requires 5 min to give positive results. Recently, however, issues on false negativity have been raised for the Abbott ID Now because of its relatively high LOD.<sup>59</sup> This may be attributed to the compromised performance of the RdRP target<sup>60,61</sup> used in this assay, which is found to be mutating and evolving.<sup>62</sup>

Rolling circle amplification (RCA)<sup>63</sup> 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.<sup>64</sup> Compared to the LAMP assay, the RCA method is simpler since it requires fewer steps and can be performed at room temperature. The method offers high sensitivity comparable to RT-PCR<sup>65</sup> since it amplifies the target sequence by  $\sim 10,000$ . 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.<sup>65</sup> 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 DNA circularization. Inappropriate design of complementary sequences therefore results in 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 manufacture, and full compatibility with POCT, which allow 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.<sup>66–68</sup> 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. Rebut et al.<sup>68</sup> 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.<sup>68</sup> 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.<sup>69</sup>

Although nucleic acid based lateral flow assays are sensitive, lateral flow immunoassays have gained interest in the massive surveillance of the COVID-19 pandemic because of their

simplicity and cheap cost. Currently, IgM/IgG rapid test kits are available for qualitative antibody testing 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.<sup>66</sup> 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.<sup>70</sup> Other signal amplification strategies include solvent evaporation for analyte pre-concentrations, nanoparticle catalyzed nanoparticle labeled assays, and ion concentration polarization methods.<sup>71</sup>

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

**Other Emerging Methods.** As discussed in *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 *Isothermal Amplification for Nucleic Acid Targets* and *Lateral Flow Based Detection of Nucleic Acid Targets* sections, other emerging methods have been developed to improve these features. To improve the sensitivity, methods with single-molecule detection capability can be used.<sup>76–78</sup> As an example, the single molecule enzyme linked immunosorbent assay (ELISA) has been developed to offer a detection limit of subfemtomolar protein concentrations.<sup>79</sup> 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 applicable in clinical setting, however, this method requires special equipment, increasing its cost.

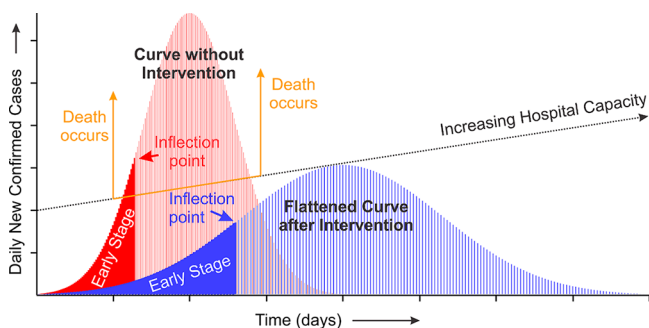
To increase the specificity, Proximity Ligation Assay (PLA) has been developed. The method utilizes two or more DNA-tagged aptamers or antibodies for binding of multiple targets.<sup>80</sup> The DNA tags on the probes are amplified only when the two different targets are in close proximity. The multiple targets ensure the specificity of target detection. However, this method requires intact SARS-CoV-2 virus particles from which two different targets are present for positive detection. This demands stringent sample processing steps.

To increase the throughput, fast sequencing such as next generation sequencing<sup>81</sup> and DNA microarray<sup>82</sup> can be used. In the case of COVID-19, evidence has 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.<sup>83</sup> The DNA microarray has been used in high-throughput identification of mutations in SARS-CoV-2.<sup>84</sup> 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 [Background](#), 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](#),



**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 time to increase the hospital capacity, each of which reduces the overall mortality rate.

intervention such as identification of patients for isolation at the early stage before the inflection point of 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 reduce 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

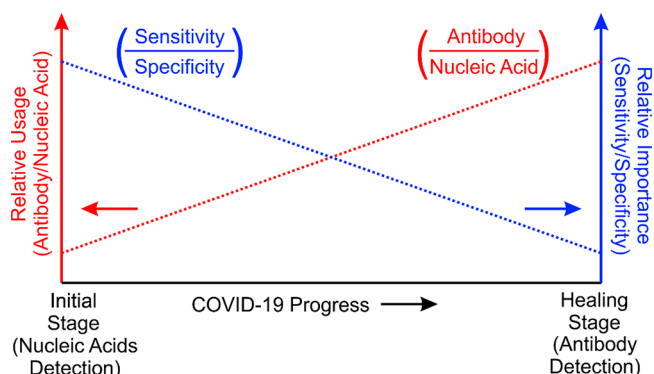
**Table 3.** Kits Based on “Not Identified” Mechanism<sup>a</sup>

Authorization	Manufacturer <sup>b</sup>	Mechanism	Target	LOD	Time
-	Biological Technologies Co., LTD	-	-	-	-
-	(Chongqing) Biotech, Co., Ltd.	-	-	-	-
-	Health Technology Co., Ltd.	-	-	-	-
-	Biotech Co., Ltd.	-	-	-	-
-	Medical Technology Co., Ltd.	-	-	-	-
-	Biotechnologies (Hangzhou) Ltd.	-	-	-	-
-	Biomedicine Co., Ltd.	-	-	-	-

<sup>a</sup>Data Not Available <sup>b</sup>Reference for kits: <http://ph.china-embassy.org/eng/sgdt/t1760281.htm>

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<sup>85,86</sup> of different assays in the fight against the COVID-19 pandemic.

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



**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.

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 aM (for a reaction volume of 100  $\mu$ L). It is noteworthy that high sensitivity is often accompanied by increased false positive results.<sup>87,88</sup> At the beginning of a viral outbreak, however, some false positive level may be tolerated. Since there are not as 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 infection concerns.

Due to the extensive amplifications, isothermal amplification-based methods<sup>89</sup> (see [Table 1](#)) usually have superior sensitivities, albeit with increased false positive levels<sup>87,88</sup> (see [Isothermal Amplification for Nucleic Acid Targets](#) section). 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 [Chest CT Imaging](#) section, 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 overburdened 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 Chinese 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,<sup>14</sup> 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 numbers of nucleic acid based kits to test all suspicious cases. The 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 have lasted about 1 week. Since these immunoassays are cheaper, faster, and easier to perform<sup>44</sup> with respect to nucleic acid based methods, they can be quickly and massively conducted by staff at drive-through 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 reset to normal ([Figure 4](#)).

In the stage where 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 COVID-19, and therefore immune to the SARS-CoV-2 virus. Since these people are clear of viral load, only an 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 are therefore vulnerable to COVID-19, are wrongly identified as immune to the disease. This misidentification will expose them to SARS-CoV-2 infection, which increases the chance for recurrence of COVID-19 in a recovering society.

In the future, affordable POC testing (POCT) kits as discussed in the [Ideal Characteristics of Diagnostic Methods](#) section may present a viable direction to address the bottleneck diagnosis problem caused by a shortage of testing kits. These kits

can be used at home for self-isolated people with mild symptoms. If they test positive using 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 the pandemic. We therefore name such an approach the massive POCT strategy. Given that 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 detection.

## ■ CONCLUSIONS AND PERSPECTIVES

In summary, like other viruses in the Panic Zone, SARS-CoV-2 has caused unexpected damage to society. During the outbreak of COVID-19, most studies have focused on the potential causes and epidemiology of the virus, while the information on 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 a 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 COVID-19 and the features of the 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 lockdown stage. Although nucleic acid based detection kits, RT-PCR in particular, offer the best solutions so far to these requirements because of their high sensitivity and specificity, immunoassays can effectively supplement the detection armory due to their cheaper price, simpler operation, and faster detection time. Immunoassays are especially useful at the later stages of the virus outbreak when people who have recovered from 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 entire 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 these so-called massive POCT kits for Panic Zone viruses therefore should be encouraged both by the government and by the private sector.

## ■ AUTHOR INFORMATION

### Corresponding Author

Hanbin Mao – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44240, United States; [orcid.org/0000-0002-6720-9429](https://orcid.org/0000-0002-6720-9429); Phone: (+1) 330-672 9380 850; Email: [hmao@kent.edu](mailto:hmao@kent.edu)

### Authors

Pravin Pokhrel – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44240, United States; [orcid.org/0000-0003-4259-6087](https://orcid.org/0000-0003-4259-6087)

Changpeng Hu – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44240, United States; Department of Pharmacy, The Second Affiliated Hospital, Army Medical University, Chongqing 400037, China

Complete contact information is available at:  
<https://pubs.acs.org/10.1021/acssensors.0c01153>

### Author Contributions

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## 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.

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