

# Diagnosing COVID-19: The Disease and Tools for Detection

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Cite This: <https://dx.doi.org/10.1021/acsnano.0c02624>



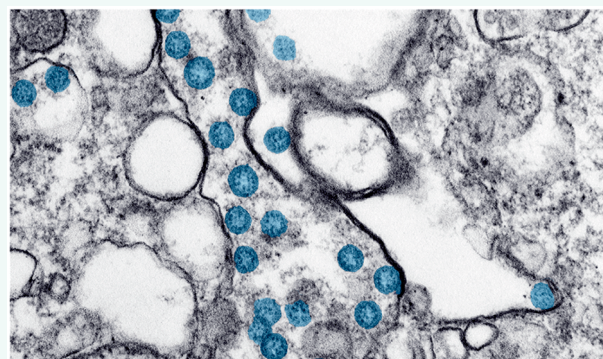
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**ABSTRACT:** COVID-19 has spread globally since its discovery in Hubei province, China in December 2019. A combination of computed tomography imaging, whole genome sequencing, and electron microscopy were initially used to screen and identify SARS-CoV-2, the viral etiology of COVID-19. The aim of this review article is to inform the audience of diagnostic and surveillance technologies for SARS-CoV-2 and their performance characteristics. We describe point-of-care diagnostics that are on the horizon and encourage academics to advance their technologies beyond conception. Developing plug-and-play diagnostics to manage the SARS-CoV-2 outbreak would be useful in preventing future epidemics.



**KEYWORDS:** SARS-CoV-2, diagnostics, COVID-19, PCR, surveillance, pandemic

Coronavirus disease 2019 (COVID-19) was discovered in Hubei Province, China in December 2019.<sup>1</sup> A cluster of patients were admitted with fever, cough, shortness of breath, and other symptoms.<sup>2</sup> Patients were scanned using computed tomography (CT), which revealed varied opacities (denser, more profuse, and confluent) in comparison to images of healthy lungs.<sup>3</sup> This finding led to the initial diagnosis of pneumonia. Additional nucleic acid analysis using multiplex real-time polymerase chain reaction (PCR) of known pathogen panels led to negative results, suggesting that the cause of pneumonia was of unknown origin.<sup>1</sup> By January 10, 2020, samples from patients' bronchoalveolar lavage (BAL) fluid were analyzed to reveal a pathogen with a similar genetic sequence to the betacoronavirus B lineage. It was discovered that this new pathogen had ~80%, ~50%, and ~96% similarity to the genome of the severe acute respiratory syndrome virus (SARS-CoV), Middle East respiratory syndrome virus (MERS-CoV), and bat coronavirus RaTG13, respectively.<sup>1,4</sup> The novel coronavirus was named SARS-CoV-2, the pathogen causing COVID-19. As of April 2, 2020, the disease has spread to at least 202 countries, infected over 1 million people, and resulted in at least 45,526 deaths globally. It is suspected that the total number of reported COVID-19 infections is underestimated, as there are many mild or asymptomatic cases that go undetected.<sup>5</sup> From the Diamond Princess cruise ship case

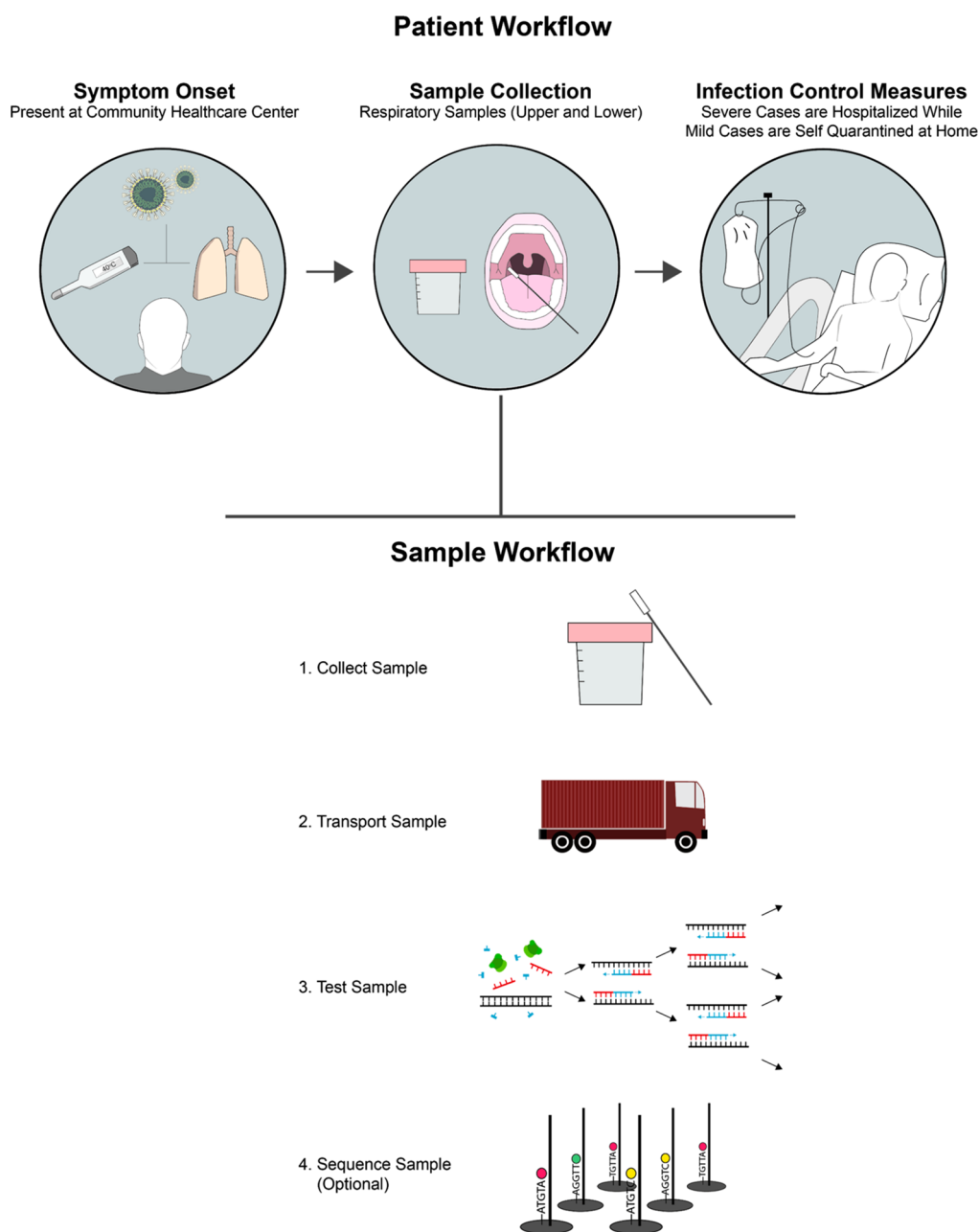
study, an estimate of 17.9% of asymptomatic cases were reported. Asymptomatic individuals are as infectious as symptomatic individuals and are therefore capable of further spreading the disease.<sup>6</sup>

SARS-CoV-2 can be transmitted from human to human. The current hypothesis is that the first transmission occurred between bats and a yet-to-be-determined intermediate host animal.<sup>1</sup> It is estimated that a SARS-CoV-2-infected person will infect approximately three new people (the reproductive number is averaged to be 3.28).<sup>7</sup> The symptoms can vary, with some patients remaining asymptomatic, while others present with fever, cough, fatigue, and a host of other symptoms. The symptoms may be similar to patients with influenza or the common cold. At this stage, the most likely mode of transmission is thought to be through direct contact and droplet spread.<sup>8,9</sup> A recent study looking at aerosol and surface stability of SARS-CoV-2 showed that the virus may be found in aerosols (<5  $\mu\text{m}$ ) for at least up to 3 h and may be

**Received:** March 27, 2020

**Accepted:** March 30, 2020

**Published:** March 30, 2020



**Figure 1.** Example of patient and sample workflow during the COVID-19 outbreak. Patients present at a healthcare facility for triage. The collected samples are tested on-site if possible or transported for molecular testing and sequencing. Patients are then managed appropriately.

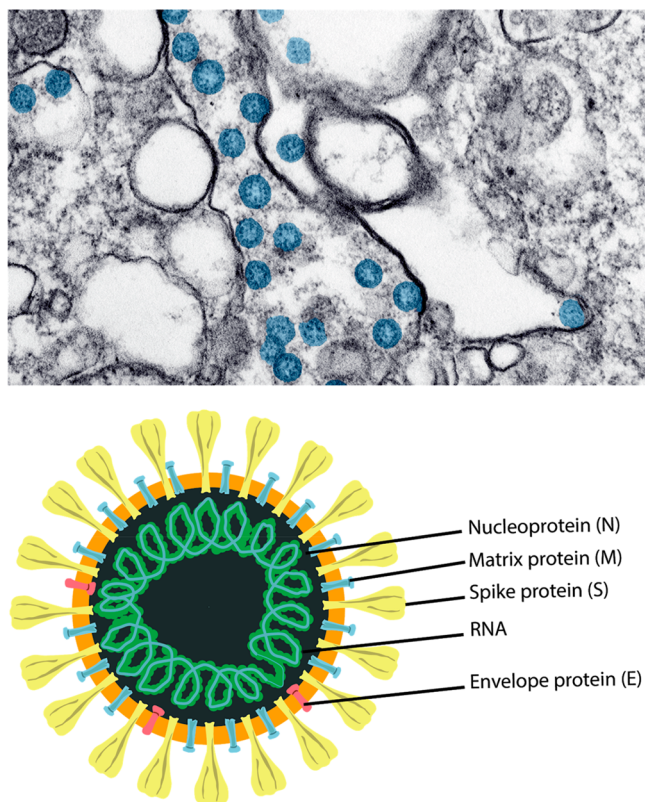
more stable on plastic and stainless steel than on copper and cardboard.<sup>10</sup>

Development of therapeutics and vaccines is underway, but there are currently no United States Food and Drug Administration (FDA) approved therapeutics or vaccines for the treatment of COVID-19 patients.<sup>11,12</sup> Diagnostics can play an important role in the containment of COVID-19, enabling the rapid implementation of control measures that limit the spread through case identification, isolation, and contact tracing (*i.e.*, identifying people that may have come in contact with an infected patient). The current diagnostic workflow for COVID-19 is described in Figure 1. In this review article, we aim to summarize the current known biological properties of SARS-CoV-2, diagnostic tools and clinical results for detecting SARS-CoV-2, emerging diagnostics, and surveillance technology to curb the spread. This is a rapidly moving topic of

research, and a review article that encompasses the current findings may be useful for guiding strategies to deal with the current COVID-19 pandemic.

**Biological Properties of SARS-CoV-2.** SARS-CoV-2 was first identified from patient samples in Wuhan, China. Human airway epithelial cells were cultured with the virus from BAL fluid isolated from patients. Supernatant was collected from cells that were damaged or killed and analyzed by negative-stained transmission electron microscopy (Figure 2).<sup>13</sup> The images revealed that the virus has a diameter ranging from 60 to 140 nm, has an envelope with protein spikes, and has genetic material.<sup>14</sup> The overall structure looks similar to other viruses from the *Coronaviridae* family.

SARS-CoV-2 has a single-stranded positive sense RNA genome that is ~30,000 nucleotides in length.<sup>1,15</sup> The genome encodes 27 proteins including an RNA-dependent RNA



**Figure 2. SARS-CoV-2 morphology.** Transmission electron microscope image of SARS-CoV-2 spherical viral particles in a cell.<sup>13</sup> The virus is colorized in blue (adapted from the US Centers for Disease Control). Representation of the viral structure is illustrated with its structural viral proteins.

polymerase (*RdRP*) and four structural proteins.<sup>15,16</sup> *RdRP* acts in conjunction with nonstructural proteins to maintain genome fidelity. A region of the *RdRP* gene in SARS-CoV-2 was shown to be highly similar to a region of the *RdRP* gene found in bat coronavirus RaTG13 and 96% similar to the RaTG13 overall genome sequence.<sup>1</sup> Of 104 strains sequenced between December 2019 and mid-February 2020, 99.9% sequence homology was observed, but, more recently, changes in the viral genome have been catalogued, showing a higher sequence diversity.<sup>2,17</sup>

The four structural proteins of SARS-CoV-2 include the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N). In coronaviruses, the *S* gene codes for the receptor-binding spike protein that enables the virus to infect cells.<sup>18</sup> This spike protein mediates receptor binding and membrane fusion, which determines host tropism and transmission capabilities.<sup>4</sup> In SARS-CoV-2, the *S* gene is divergent with <75% nucleotide sequence similarity when compared to all previously described SARS-related coronaviruses.<sup>1</sup> The other three structural proteins are more conserved than the spike protein and are necessary for general coronavirus function.<sup>15</sup> These proteins are involved in encasing the RNA and/or in protein assembly, budding, envelope formation, and pathogenesis.<sup>19–21</sup>

SARS-CoV-2 appears to interact with the angiotensin converting enzyme 2 (ACE2) receptor for entry into cells. Zhou *et al.* conducted infectivity studies by incubating SARS-CoV-2 with HeLa cells of differential ACE2 receptor expression.<sup>1</sup> The authors showed co-localization of the

fluorescently stained viruses with cells that express the ACE2 receptor from Chinese horseshoe bats, pigs, humans, and civets, but not from mice. The ACE2 mRNA is present in almost all human organs. ACE2 is present in arterial and venous endothelial cells and arterial smooth muscle cells in the lungs, stomach, small intestine, colon, skin, lymph nodes, liver bile ducts, kidney parietal epithelial cells, and the brain. It is also expressed on the surface of lung alveolar epithelial cells and enterocytes of the small intestine that allows them to be infected. Tissues of the upper respiratory tract (*i.e.*, oral and nasal mucosa and nasopharynx) did not show surface expression of ACE2 on epithelial cells and therefore are unlikely the primary site of SARS-CoV-2 infection.<sup>22</sup> CT scans may show higher opacity in the lower lungs because cells in that region express more ACE2. SARS-CoV-2 has been isolated from oral swabs, BAL fluid, and stool.<sup>1,23</sup> Higher viral loads have been recorded in the nose *versus* the throat, with similar viral loads seen in asymptomatic and symptomatic patients.<sup>24</sup> Understanding the biological properties of SARS-CoV-2 enabled researchers to develop diagnostics for detection.

**Current Diagnostic Tests for COVID-19.** The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis. Guan *et al.* reported that 44% of 1099 COVID-19 patients from China had a fever when they entered the hospital and that 89% developed a fever while in hospital.<sup>25</sup> They further found that patients had a cough (68%), fatigue (38%), sputum production (34%), and shortness of breath (19%). Many of these symptoms could be associated with other respiratory infections. Nucleic acid testing and CT scans have been used for diagnosing and screening COVID-19.

Molecular techniques are more suitable than syndromic testing and CT scans for accurate diagnoses because they can target and identify specific pathogens. The development of molecular techniques is dependent upon understanding (1) the proteomic and genomic composition of the pathogen or (2) the induction of changes in the expression of proteins/genes in the host during and after infection. As of March 24, 2020, the genomic and proteomic compositions of SARS-CoV-2 have been identified, but the host response to the virus is still under investigation. The first genome sequence of SARS-CoV-2 was conducted with metagenomic RNA sequencing, an unbiased and high-throughput method of sequencing multiple genomes.<sup>26–28</sup> The findings were publicly disclosed, and the sequence was added to the GenBank sequence repository on January 10, 2020.<sup>26,27</sup> Since then, more than 1000 sequences have been made available on the Global Initiative on Sharing All Influenza Data (GISAID) and GenBank by researchers across the globe.<sup>29,30</sup> According to the joint report by the World Health Organization (WHO) and China, 104 strains of the SARS-CoV-2 virus were isolated and sequenced using Illumina and Oxford nanopore sequencing from the end of December 2019 to mid-February 2020.<sup>2,4</sup> Illumina sequencing is a sequence-by-synthesis method using solid-phase bridge amplification, whereas nanopore sequencing involves translocating a DNA molecule through a protein pore and measuring subsequent shifts in voltage to determine the DNA sequence.<sup>31</sup> Genome sequencing is important for researchers to design primers and probe sequences for PCR and other nucleic acid tests.

**Nucleic Acid Testing. Designing a Nucleic Acid Test for SARS-CoV-2.** Nucleic acid testing is the primary method of

Table 1. Polymerase Chain Reaction (PCR) Tests/Primers for SARS-CoV-2

Institution	Gene target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')
U.S. CDC <sup>32</sup>	N gene	<b>N1:</b> GACCCAAAATCAGCGAAAT  <b>N2:</b> TTACAAACATTGGCCGCAA  <b>N3:</b> GGGAGCCTTGAATACACAAAA  <b>RP-F RNase:</b> AGATTTGGACCTGCGAGCG	<b>N1:</b> TCTGGTACTGCCAGTTGAATCTG  <b>N2:</b> GCGCGACATTCCGAAGAA  <b>N3:</b> TGTAGCACGATTGCAGCATTG  <b>RP-RRNase:</b> GAGCGGCTGTCTCCACAAGT	<b>N1:</b> FAM-ACCCCGCATTACGTTTGTGGACC-BHQ1  <b>N2:</b> FAM-ACAATTTGCCCCAGCGCTTCAG-BHQ1  <b>N3:</b> FAM-AYCACATTGGCACCCGCAATCCTG-BHQ1  <b>RP-P RNase:</b> FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ-1
China CDC <sup>36</sup>	ORF1ab and N gene	<b>ORF1ab:</b> CCCTGTGGGTTTACACTTAA  <b>N:</b> GGGAACTTCTCCTGCTAGAAT	<b>ORF1ab:</b> ACGATTGTGCATCAGCTGA  <b>N:</b> CAGACATTTTGTCTCAAGCTG	<b>ORF1ab:</b> FAM-CCGCTGCGGTATGTGGAAAGGTTATGG-BHQ1  <b>N:</b> FAM-TTGTCTGCTGCTTGA CAGATT-TAMRA
Charité, Germany <sup>43</sup>	RdRp, E, N gene	<b>RdRp:</b> GTGARATGGTCATGTGTGGCGG  <b>E:</b> ACAGGTACGTTAATAGTTAATAGCGT	<b>RdRp:</b> CARATGTAAASACACTATTAGCATA  <b>E:</b> ATATTGCAGCAGTACGCACACA	<b>RdRp 1:</b> FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ  <b>RdRp 2:</b> FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ  <b>E:</b> FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
Hong Kong University <sup>36</sup>	ORF1b-nsp14, N gene	<b>ORF1b-nsp14:</b> TGGGGYTTTACRGGTAACCT  <b>N:</b> TAATCAGACAAGGAACTGATTA	<b>ORF1b-nsp14:</b> AACRCGCTTAACAAAGCACTC  <b>N:</b> CGAAGGTGTGACTTCCATG	<b>ORF1b-nsp14:</b> FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA  <b>N:</b> FAM-GCAAATTTGTGCAATTTGCGG-TAMRA
National Institute of Infectious Diseases, Japan <sup>37</sup>	N gene	<b>N:</b> AAATTTGGGGACCAGGAAC	<b>N:</b> TGGCAGCTGTGTAGGTCAAC	<b>N:</b> FAM-ATGTCGCGCATTGGCATGGA-BHQ
National Institute of Health, Thailand <sup>38</sup>	N gene	<b>N:</b> CGTTTGGTGGACCCTCAGAT	<b>N:</b> CCCCACTGCGTTCTCCATT	<b>N:</b> FAM-CAACTGGCAGTAACCBQHI

diagnosing COVID-19.<sup>32</sup> A number of reverse transcription polymerase chain reaction (RT-PCR) kits have been designed to detect SARS-CoV-2 genetically (Table 1). RT-PCR involves the reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the cDNA.<sup>33,34</sup> The design process generally involves two main steps: (1) sequence alignment and primer design, and (2) assay optimization and testing. Corman *et al.* aligned and analyzed a number of SARS-related viral genome sequences to design a set of primers and probes.<sup>35</sup> Among the SARS-related viral genomes, they discovered three regions that had conserved sequences: (1) the *RdRP* gene (RNA-dependent RNA polymerase gene) in the open reading frame ORF1ab region, (2) the *E* gene (envelope protein gene), and (3) the *N*

gene (nucleocapsid protein gene). Both the *RdRP* and *E* genes had high analytical sensitivity for detection (technical limit of detection of 3.6 and 3.9 copies per reaction), whereas the *N* gene provided poorer analytical sensitivity (8.3 copies per reaction). The assay can be designed as a two-target system, where one primer universally detects numerous coronaviruses including SARS-CoV-2 and a second primer set only detects SARS-CoV-2.

After designing the primers and probes, the next step involves optimizing assay conditions (*e.g.*, reagent conditions, incubation times, and temperatures), followed by PCR testing. RT-PCR can be performed in either a one-step or a two-step assay. In a one-step assay, reverse transcription and PCR amplification are consolidated into one reaction. This assay

format can provide rapid and reproducible results for high-throughput analysis. The challenge is the difficulty in optimizing the reverse transcription and amplification steps as they occur simultaneously, which leads to lower target amplicon generation. In the two-step assay, the reaction is done sequentially in separate tubes.<sup>36</sup> This assay format is more sensitive than the one-step assay, but it is more time-consuming and requires optimizing additional parameters.<sup>36,37</sup> Lastly, controls need to be carefully selected to ensure the reliability of the assay and to identify experimental errors.

**Workflow for Nucleic Acid Testing for SARS-CoV-2.** At least 11 nucleic-acid-based methods and eight antibody detection kits have been approved in China by the National Medical Products Administration (NMPA) for detecting SARS-CoV-2.<sup>38</sup> However, RT-PCR is the most predominantly used method for diagnosing COVID-19 using respiratory samples.<sup>2,39</sup> Upper respiratory samples are broadly recommended, although lower respiratory samples are recommended for patients exhibiting productive cough.<sup>40</sup> Upper respiratory tract samples include nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal washes, and nasal aspirates. Lower respiratory tract samples include sputum, BAL fluid, and tracheal aspirates. Both BAL and tracheal aspirates can be high risk for aerosol generation. The detectable viral load depends on the days after illness onset. In the first 14 days after onset, SARS-CoV-2 could most reliably be detected in sputum followed by nasal swabs, whereas throat swabs were unreliable 8 days after symptom onset.<sup>41,42</sup> Given the variability in the viral loads, a negative test result from respiratory samples does not rule out the disease. These negatives could result from improper sampling techniques, low viral load in the area sampled, or mutations in the viral genome.<sup>3,43</sup> Winichakoon *et al.* recommended multiple lines of evidence for patients linked epidemiologically even if the results are negative from nasopharyngeal and/or oropharyngeal swab.<sup>43</sup>

The United States Centers for Disease Control and Prevention (CDC) uses a one-step real time RT-PCR (rRT-PCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-CoV-2.<sup>44</sup> To perform the assay, the viral RNA is extracted and added to a master mix. The master mix contains nuclease-free water, forward and reverse primers, a fluorophore-quencher probe, and a reaction mix (consisting of reverse transcriptase, polymerase, magnesium, nucleotides, and additives).<sup>32</sup> The master mix and extracted RNA are loaded into a PCR thermocycler, and the incubation temperatures are set to run the assay. The CDC has recommended cycling conditions for rRT-PCR.<sup>44</sup> During rRT-PCR, the fluorophore-quencher probe is cleaved, generating a fluorescent signal. The fluorescent signal is detected by the thermocycler, and the amplification progress is recorded in real time. The probe sequence used by Guan *et al.* was Black Hole Quencher-1 (BHQ1, quencher) and fluorescein amidite (FAM, fluorophore). This reaction takes ~45 min and can occur in a 96-well plate, where each well contains a different sample or control. There must be both a positive and a negative control to interpret the final results properly when running rRT-PCR. For SARS-CoV-2, the CDC provides a positive control sequence called nCoVPC.<sup>44</sup> A number of SARS-CoV-2 RT-PCR primers and probes from different research groups and agencies are listed in Table 1.

**Integrating the Workflow for Nucleic Acid Detection with Clinical Management.** There are different implementation workflows for RT-PCR tests in clinical settings. Corman *et al.*

proposed a three-step workflow for the diagnosis of SARS-CoV-2.<sup>45</sup> They define the three steps as first line screening, confirmation, and discriminatory assays. To maximize the number of infected patients identified, the first step detects all SARS-related viruses by targeting different regions of the *E* gene. If this test is positive, then they propose the detection of the *RdRP* gene using two different primers and two different probes. If these results are also positive, then they conduct the discriminatory test with one of the two probe sequences.<sup>45</sup> See Table 1 (Charité, Germany). Chu *et al.* proposed a slightly different assay workflow.<sup>46</sup> They screened samples using primers for the *N* gene and used those from the *ORF1b* gene for confirmation. A diagnosis where the patient sample is positive with *N* gene primer and negative with the *ORF1b* gene would be inconclusive. In such situations, protein tests (*i.e.*, antibody tests) or sequencing would be required to confirm the diagnosis.<sup>46</sup>

**Computed Tomography.** Due to the shortage of kits and false negative rate of RT-PCR, the Hubei Province, China temporarily used CT scans as a clinical diagnosis for COVID-19.<sup>47</sup> Chest CT scans are non-invasive and involve taking many X-ray measurements at different angles across a patient's chest to produce cross-sectional images.<sup>48,49</sup> The images are analyzed by radiologists to look for abnormal features that can lead to a diagnosis.<sup>48</sup> The imaging features of COVID-19 are diverse and depend on the stage of infection after the onset of symptoms. For example, Bernheim *et al.* saw more frequent normal CT findings (56%) in the early stages of the disease (0–2 days)<sup>50</sup> with a maximum lung involvement peaking at around 10 days after the onset of symptoms.<sup>51</sup> The most common hallmark features of COVID-19 include bilateral and peripheral ground-glass opacities (areas of hazy opacity)<sup>52</sup> and consolidations of the lungs (fluid or solid material in compressible lung tissue).<sup>50,51</sup> De Wever *et al.* found that ground-glass opacities are most prominent 0–4 days after symptom onset. As a COVID-19 infection progresses, in addition to ground-glass opacities, crazy-paving patterns (*i.e.*, irregular-shaped paved stone pattern) develop,<sup>51</sup> followed by increasing consolidation of the lungs.<sup>50,51</sup> Based on these imaging features, several retrospective studies have shown that CT scans have a higher sensitivity (86–98%) and improved false negative rates compared to RT-PCR.<sup>3,25,53,54</sup> The main caveat of using CT for COVID-19 is that the specificity is low (25%) because the imaging features overlap with other viral pneumonia.<sup>3</sup>

COVID-19 is currently diagnosed with RT-PCR and has been screened for with CT scans, but each technique has its own drawbacks. There are three issues that have arisen with RT-PCR. First, the availability of PCR reagent kits has not kept up with demand. Second, community hospitals outside of urban cities lack the PCR infrastructure to accommodate high sample throughput. Lastly, RT-PCR relies on the presence of detectable SARS-CoV-2 in the sample collected. If an asymptomatic patient was infected with SARS-CoV-2 but has since recovered, PCR would not identify this prior infection, and control measures would not be enforced. Meanwhile, CT systems are expensive, require technical expertise, and cannot specifically diagnose COVID-19. Other technologies need to be adapted to SARS-CoV-2 to address these deficiencies.

**Emerging Diagnostic Tests for COVID-19.** According to the WHO, the immediate priority for COVID-19 diagnostics research is the development of nucleic acid and protein tests and detection at the point-of-care.<sup>2</sup> The longer-term priority is

to integrate these tests into multiplex panels. In order to improve surveillance efforts, serological tests using proteins are needed in addition to nucleic acid tests. These tests have the benefits of detection after recovery, unlike nucleic acid tests. This enables clinicians to track both sick and recovered patients, providing a better estimate of total SARS-CoV-2 infections. Point-of-care tests are cost-effective, hand-held devices used to diagnose patients outside of centralized facilities. These can be operated in areas like community centers to reduce the burden on clinical laboratories.<sup>55</sup>

**Nucleic Acid Testing.** Nucleic acid tests using isothermal amplification are currently in development for SARS-CoV-2 detection. Isothermal amplification techniques are conducted at a single temperature and do not need specialized laboratory equipment to provide similar analytical sensitivities to PCR.<sup>56</sup> These techniques include recombinase polymerase amplification, helicase-dependent amplification, and loop-mediated isothermal amplification (LAMP). Several academic laboratories have developed and clinically tested reverse transcription LAMP (RT-LAMP) tests for SARS-CoV-2.<sup>57–60</sup> RT-LAMP uses DNA polymerase and four to six primers to bind to six distinct regions on the target genome. In a four-primer system, there are two inner primers (a forward and a reverse inner primer) and two outer primers; LAMP is highly specific because it uses a higher number of primers.<sup>61</sup> In LAMP diagnostic tests, a patient sample is added to the tube, and the amplified DNA is detected by turbidity (a byproduct of the reaction), color (addition of a pH-sensitive dye), or fluorescence (addition of a fluorescent dye that binds to double-stranded DNA).<sup>62</sup> The reaction occurs in <1 h at 60–65 °C with an analytical limit of detection of ~75 copies per  $\mu\text{L}$ . The approach is simple to operate, easy to visualize for detection, has less background signal, and does not need a thermocycler.<sup>61</sup> The drawbacks to LAMP are the challenges of optimizing primers and reaction conditions. Other isothermal amplification techniques for COVID-19 detection are in development.<sup>62</sup>

Isothermal amplification techniques can be multiplexed at the amplification and/or readout stage. Multiplexing can use polymeric beads encoded with unique optical signatures (e.g., organic fluorescent molecules) for barcoding. Barcodes can be designed for different biomarkers in panels to detect multiple analytes from a single patient sample in one reaction tube.<sup>63</sup> Multiplexing increases the amount of information gained from a single test and improves clinical sensitivity and specificity.<sup>64</sup> One way of encoding unique signatures is through agents that emit fluorescent signals. Each unique emission codes for the capturing DNA or antibody on the bead surface. A positive detection occurs when a patient's sample contains a sequence or antigen that links the bead's capture molecule with a secondary probe (labeled with fluorophore with a different emission than the beads). There are barcoded-bead multiplex panels for diagnosing cystic fibrosis and respiratory diseases.<sup>65,66</sup> Barcoded-bead assays/systems are engineered for laboratory use, but efforts are underway to develop them for the point-of-care. However, the difficulty lies with the design of the readout device. The complex barcode signal, which stems from the organic molecules, requires a unique instrument design to discern the codes. Researchers are working on overcoming this limitation by using inorganic quantum dots for barcoding, which enables battery-operated excitation and a smartphone camera to capture the emission signal. Kim *et al.* have demonstrated clinical specificity and sensitivity of 91%

and 95% using quantum dot barcodes for diagnosing patients with hepatitis B after isothermal reverse polymerase amplification.<sup>63</sup> Panels of barcodes can be formulated into tablets for easy dissemination of reagents.<sup>67</sup> Barcode panels can be designed for respiratory viruses, coronaviruses, sexually transmitted diseases, and/or symptoms (e.g., fever, cough, diarrhea).

In addition to isothermal amplification, there are other nucleic acid tests that could be used for SARS-CoV-2 detection. SHERLOCK is a detection strategy that uses Cas13a ribonuclease for RNA sensing.<sup>68</sup> Viral RNA targets are reverse transcribed to cDNA and isothermally amplified using reverse polymerase amplification. The amplified products are transcribed back into RNA. Cas13a complexes with a RNA guide sequence that binds with the amplified RNA product.<sup>69</sup> Upon target binding, Cas13a is activated. Cas13a then cleaves surrounding fluorophore-quencher probes to produce a fluorescent signal. All components of SHERLOCK can be freeze-dried. Prior studies using SHERLOCK could detect as few as 2000 copies/mL in clinical serum or urine isolates for Zika virus.<sup>70</sup> A SHERLOCK protocol for detecting SARS-CoV-2 has been released,<sup>71</sup> and another Cas13a-based detection system has been tested with SARS-CoV-2 clinical isolates.<sup>72</sup>

**Protein Testing.** Viral protein antigens and antibodies that are created in response to a SARS-CoV-2 infection can be used for diagnosing COVID-19. Changes in viral load over the course of the infection may make viral proteins difficult to detect. For example, Lung *et al.* showed high salivary viral loads in the first week after onset of symptoms, which gradually declined with time.<sup>73</sup> In contrast, antibodies generated in response to viral proteins may provide a larger window of time for indirectly detecting SARS-CoV-2. Antibody tests can be particularly useful for surveillance of COVID-19. One potential challenge with developing accurate serological tests includes potential cross-reactivity of SARS-CoV-2 antibodies with antibodies generated against other coronaviruses. Lv *et al.* tested plasma samples from 15 COVID-19 patients against the S protein of SARS-CoV-2 and SARS-CoV and saw a high frequency of cross-reactivity.<sup>74</sup>

Currently, serological tests (i.e., blood tests for specific antibodies) are in development.<sup>75–77</sup> Zhang *et al.* detected immunoglobulin G and M (IgG and IgM) from human serum of COVID-19 patients using an enzyme-linked immunosorbent assay (ELISA).<sup>75</sup> They used the SARS-CoV-2 Rp3 nucleocapsid protein, which has 90% amino acid sequence homology to other SARS-related viruses. The recombinant proteins adsorb onto the surface of 96-well plates, and the excess protein is washed away. Diluted human serum is added for 1 h, after which the plate is washed again. Antihuman IgG functionalized with horseradish peroxidase is added and allowed to bind to the target. The plate is washed, followed by the addition of the substrate 3,3',5,5'-tetramethylbenzidine. The peroxidase reacts with the substrate to cause a color change that can be detected by a plate reader. If anti-SARS-CoV-2 IgG is present, it will be sandwiched between the adsorbed nucleoprotein and the antihuman IgG probe, resulting in a positive signal. The IgM test by Zhang *et al.* has a similar structure but uses antihuman IgM adsorbed to the plate and an anti-Rp3 nucleocapsid probe. They tested 16 SARS-CoV-2 positive patient samples (confirmed by RT-PCR) and found the levels of these antibodies increased over the first 5 days after symptom onset. On day zero, 50% and 81% of patients were positive for

Table 2. Emerging Diagnostics Being Developed for SARS-CoV-2

platform	biomarker	POC (Y/N)	type of technology	how it works	types of clinical sample	clinical sample tested
CRISPR <sup>109</sup>	nucleic acid	Y	RPA	PCR, perform CRISPR/Ca9-mediated lateral flow nucleic assay (CASLFA)	serum	110
CRISPR <sup>68</sup>	nucleic acid	Y	RT-RPA	RPA, SHERLOCK multiplexed signal detection <i>via</i> fluorescence	nasopharyngeal swabs	384
LAMP <sup>110</sup>	nucleic acid	N	LAMP	isothermal DNA synthesis using self-recurring strand displacement reactions; positive detection leads to increased sample turbidity	throat swabs	53
RPA <sup>111</sup>	nucleic acid	N	RPA	forward and reverse primers blind to DNA and amplify strands at 37 °C	fecal and nasal swabs	30
NASBA <sup>112</sup>	nucleic acid	N	real-time NASBA	transcription-based amplification for RNA targets	nasal swabs	138
RCA <sup>113</sup>	nucleic acid	N	rolling circle amplification	DNA polymerase used to extend a circular primer and repeatedly replicate the sequence	serum	7
RT-LAMP <sup>114</sup>	nucleic acid	N	LAMP	reverse transcriptase LAMP reaction for RNA targets	nasopharyngeal aspirates	59
smartphone dongle <sup>81</sup>	protein	Y	ELISA	microfluidics-based cassette operating an ELISA	blood	96
quantum dot barcode <sup>63</sup>	nucleic acid	Y	barcode	multiplexed quantum beads capture viral DNA for RPA detection	serum	72
magnetic bead <sup>115</sup>	nucleic acid	N	magnetic	magnetic beads isolate bacteria for PCR detection	stool	17
paramagnetic bead <sup>116</sup>	protein	N	magnetic biosensor	magnetic separation of protein targets	serum	12
magnetic bead isolation <sup>117</sup>	whole bacteria	N	magnetic separation	magnetic isolation of bacteria	synovia	12
ELISA <sup>118</sup>	protein	N	ELISA	enzymatic reaction to produce colored product in presence of target	serum	30
SIMOA <sup>119</sup>	protein	N	digital ELISA	digital readout of colored product by enzymatic reaction in presence of target	serum	30
biobarcode assay <sup>120</sup>	protein	N	DNA-assisted immunoassay	protein signal is indirectly detected by amplifying DNA conjugated to gold nanoparticle	serum	18
rapid antigen test <sup>121</sup>	protein	Y	lateral flow	gold-coated antibodies produce colorimetric signal on paper in presence of target	serum	117

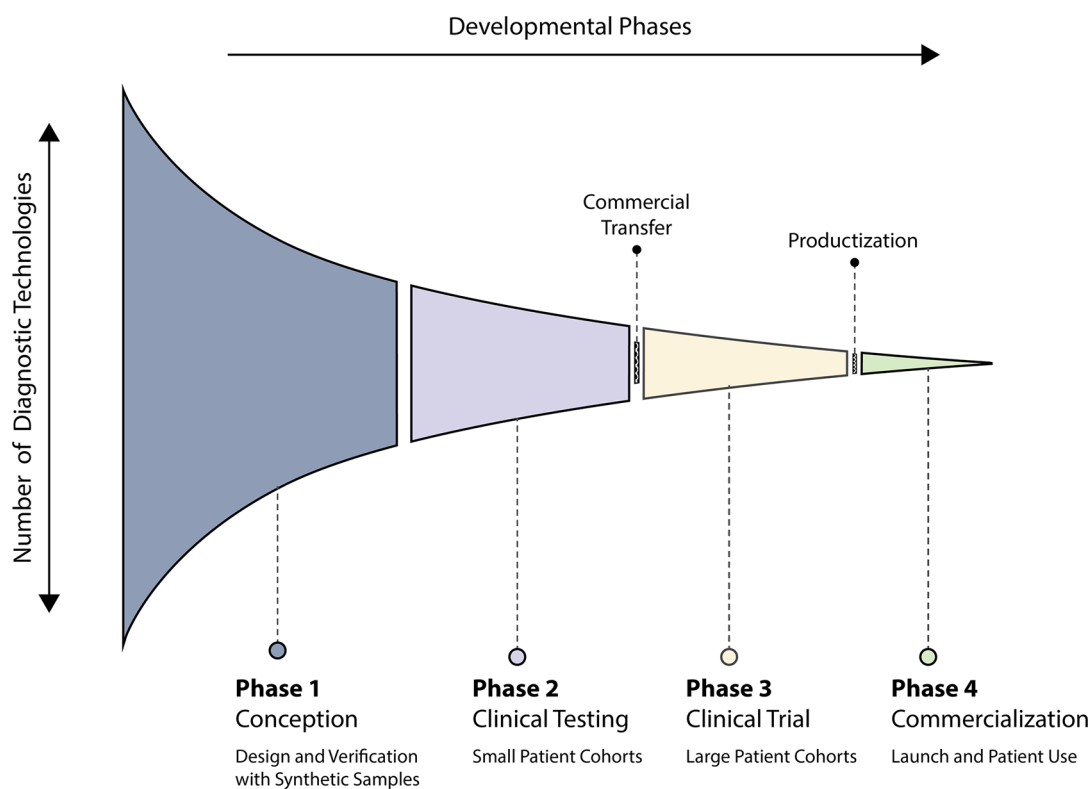
IgM and IgG, respectively, but this increased to 81% and 100% at day five.<sup>75</sup> Antibodies were detected in respiratory, blood, or fecal samples. Xiang *et al.* also detected SARS-CoV-2 IgG and IgM antibodies in suspected cases.<sup>76</sup> Given the recent studies, there may also be other protein or cellular markers that can be used for detection. Guan *et al.* showed that infected patients had elevated levels of C-reactive protein and D-dimer as well as low levels of lymphocytes, leukocytes, and blood platelets.<sup>25</sup> The challenge of using these biomarkers is that they are also abnormal in other illnesses. A multiplex test with both antibody and small molecule markers could improve specificity.

**Point-of-Care Testing.** Point-of-care tests are used to diagnose patients without sending samples to centralized facilities, thereby enabling communities without laboratory infrastructure to detect infected patients. Lateral flow antigen detection for SARS-CoV-2 is one point-of-care approach under development for diagnosing COVID-19.<sup>76</sup> In commercial lateral flow assays, a paper-like membrane strip is coated with two lines: gold nanoparticle-antibody conjugates are present in one line and capture antibodies in the other. The patient's sample (*e.g.*, blood and urine) is deposited on the membrane, and the proteins are drawn across the strip by capillary action. As it passes the first line, the antigens bind to the gold nanoparticle-antibody conjugates, and the complex flows together through the membrane. As they reach the second line, the complex is immobilized by the capture antibodies, and a red or blue line becomes visible. Individual gold nanoparticles are red in color, but a solution containing clustered gold nanoparticles is blue due to the coupling of the plasmon band. The lateral flow assay has demonstrated a

clinical sensitivity, specificity, and accuracy of 57%, 100%, and 69% for IgM and 81%, 100%, and 86% for IgG, respectively. A test that detects both IgM and IgG yields a clinical sensitivity of 82%.<sup>76</sup> Nucleic acid testing can also be incorporated into the lateral flow assay. Previously, a RT-LAMP test was combined with lateral flow readout to detect MERS-CoV.<sup>78</sup> These tests are single use and suffer from poor analytical sensitivity in comparison to RT-PCR. To improve the assay readout signal, researchers have developed a variety of signal amplifying techniques (*e.g.*, thermal imaging and assembly of multiple gold nanoparticles).<sup>79</sup>

Another approach for use at the point-of-care is microfluidic devices. These devices consist of a palm-sized chip etched with micrometer-sized channels and reaction chambers. The chip mixes and separates liquid samples using electrokinetic, capillary, vacuum, and/or other forces. These chips can be constructed with materials such as polydimethyl sulfoxide, glass, or paper. The key advantages of using microfluidics include miniaturization, small sample volume, rapid detection times, and portability.<sup>80</sup> Laksanasopin *et al.* developed a microfluidics-based smartphone attachment to detect antibodies against three sexually transmitted infections by sequentially moving reagents prestored on a cassette. The platform showed 100% and 87% clinical sensitivity and specificity for HIV, respectively, when tested on 96 patients in Rwanda.<sup>81</sup> These technologies can be adapted to detect SARS-CoV-2 RNA or proteins.

Here, we described some diagnostic technologies that have shown clinical feasibility. Table 2 provides a more extensive list of emerging technologies that can be adapted for detecting SARS-CoV-2. There are many platforms being developed in



**Figure 3. Developmental phases of diagnostic tests.** Phases 1 and 2 typically occur in an academic setting, while phases 3 and 4 occur in a company after commercial transfer. Most diagnostic technologies are at the proof-of-concept stage, and few are in phase 3 that can be quickly adapted for diagnosing pathogens in new outbreaks. The advancement of more phase 2 technologies into phase 3 would increase the number of approaches for detecting new pathogens.

academic laboratories such as electrochemical sensors, paper-based systems, and surface-enhanced Raman scattering based systems. Such approaches are in the early stages of development and cannot be used to diagnose COVID-19 immediately. One can delineate diagnostic technology development into four phases (Figure 3). Phase 1 refers to technologies that are at the proof-of-concept stage where researchers use synthetic targets to validate the concept. Phase 2 refers to technologies that have analyzed a small number of patient samples (*i.e.*, <100 samples). Phase 3 typically refers to technologies that advance to clinical trials with a large patient cohort. Phase 4 is when the technology is commercialized and used in patients. These emerging technologies could play a role in detecting future outbreaks.

#### Smartphone Surveillance of Infectious Diseases.

Controlling epidemics requires extensive surveillance, sharing of epidemiological data, and patient monitoring.<sup>82,83</sup> Healthcare entities, from local hospitals to the WHO, require tools that can improve the speed and ease of communication to manage the spread of diseases. Smartphones can be leveraged for this purpose as they possess the connectivity, computational power, and hardware to facilitate electronic reporting, epidemiological databasing, and point-of-care testing (Figure 4).<sup>84,85</sup> An exponential rise in worldwide smartphone adoption, including in sub-Saharan Africa, makes smartphones a widely accessible technology to coordinate responses during large outbreaks like COVID-19.<sup>84</sup>

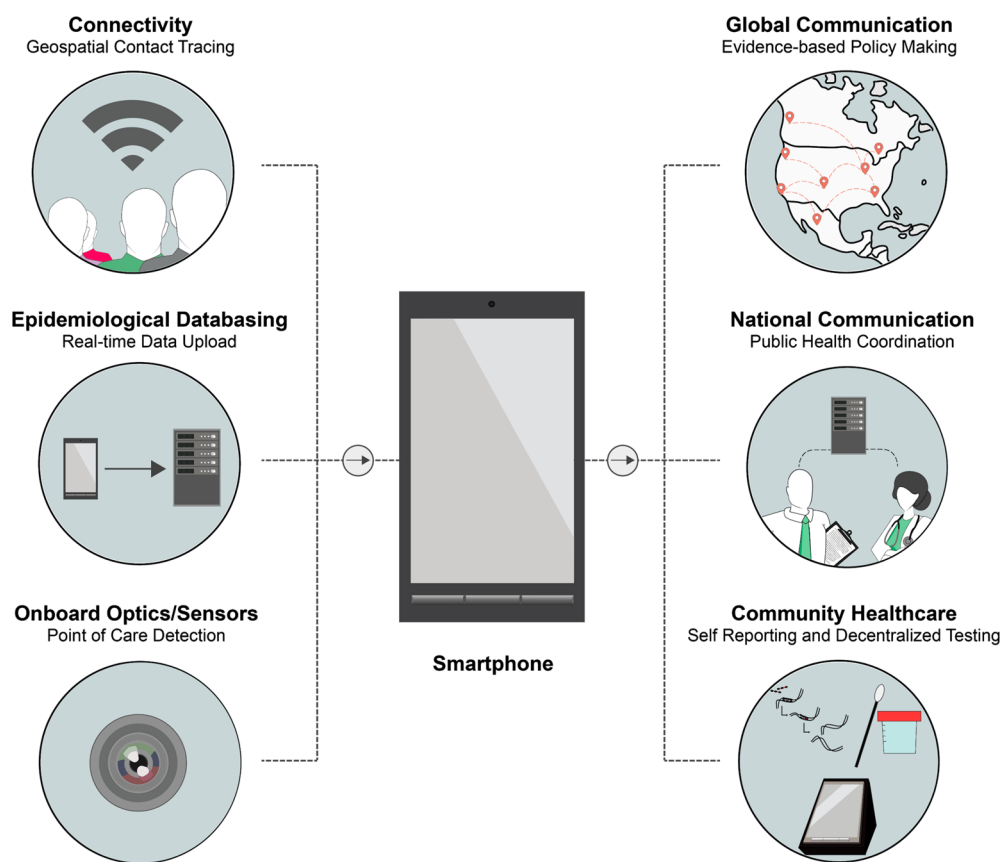
The global spread of COVID-19 has been catalyzed by insufficient communication and underreporting.<sup>86,87</sup> A notable example is Iran, which confirmed its first 43 cases by February 23, 2020, a case fatality rate of 19% (8 deaths), and 3 exported

cases of Iranian origin. From this reporting, transmission modeling suggests the number of infected individuals in Iran was in the thousands.<sup>88</sup> Smartphones can be paired with pre-existing diagnostic tests to provide real-time geospatial information that empowers national and global health agencies to implement coordinated control strategies. Several research groups have used smartphones for geospatial tracking of infectious diseases such as HIV, Ebola, and tuberculosis.<sup>89–91</sup> For Ebola, smartphones were used for contact tracing, which is the practice of tracking and identifying people that have come into contact with infected patients and may also be infected.<sup>89</sup> Smartphones can digitize the process of contact tracing to provide more complete and shareable records.

Without communication between regional healthcare agencies, transmission rates can vary across a country,<sup>92</sup> such as occurred during the 2003 SARS outbreak in Canada. Toronto, Ontario had 247 cases, 3 of which were imported, whereas Vancouver, British Columbia had only 5 cases, 4 of which were imported.<sup>92,93</sup> At the time, Ontario did not have a provincial public health agency, but British Columbia's agency previously identified that the province was at risk of importing emerging infectious diseases. Prior to the SARS outbreak, British Columbia's public health agency established a digital network to facilitate communication across the province.<sup>92</sup> These communication networks can be expanded by leveraging smartphone connectivity. Smartphones can be used in the field to upload and share epidemiological data onto public health databases and to coordinate outbreak responses.

People suspected to have COVID-19 can encounter communication barriers with their healthcare providers. Anyone exhibiting mild respiratory symptoms may be hesitant





**Figure 4. Role of smartphones in diagnostics.** Smartphone capabilities such as connectivity, databasing, and onboard hardware enable better evidence-based policy making, national disease response coordination, and community healthcare.

to travel to overcrowded hospitals, as they face an increased risk of contracting COVID-19. Smartphones can be leveraged to provide a direct line of communication between patients and clinicians without risking infection of either party. During the 2009 influenza pandemic, Switzerland used medical teleconsultations to manage suspected cases in addition to its existing reporting system.<sup>94</sup> Teleconsultations led to higher total reporting of influenza compared to in-person consultations due to the lower barrier of access. If COVID-19 infected individuals visit a hospital and test positive, patients with mild symptoms are sent home for self-quarantine.<sup>95</sup> Self-quarantine naturally deters communication between patients and clinicians, resulting in adverse mental health effects for the patient and reduced monitoring by the clinician. Smartphone apps can connect patients with mental health counselors to cope with isolation and fear during disease outbreaks and self-quarantine.<sup>96,97</sup> In addition, patients can self-report symptoms and behaviors, facilitating remote monitoring by clinicians.<sup>98</sup> Smartphone-based reporting also provides epidemiologists with information relevant to potential transmission mechanisms. For example, during the 2013 MERS outbreak, a smartphone app was used to monitor travelers during their Hajj pilgrimage. In the app, users reported hand hygiene protocols, animal contact, and onset of symptoms, both during the pilgrimage and after returning to their home countries.<sup>99</sup> Similar apps can be used to keep public health agencies actively informed and to improve responses to disease outbreaks.

In recent years, there have been significant developments in integrating smartphones and diagnostic technologies. Smartphone components (e.g., camera, flashlight, and audio jack)

have been used for the readout of diagnostic assays in place of conventional laboratory equipment.<sup>100</sup> These devices can simplify diagnostic workflow by automating readout and databasing. For example, a smartphone-based microscope was field tested in Cameroon and demonstrated faster turnaround times than standard techniques.<sup>101</sup> Kanazawa *et al.* validated the use of smartphones accompanied by forward looking infrared radar (FLIR) for the thermal detection of body temperature due to inflammation. This technology may also be adapted for the detection of fever, a common symptom of many coronaviruses including COVID-19.<sup>102</sup> Mudanyali *et al.* also developed a smartphone-based microscope that transfers diagnostic results to a database for analysis and spatiotemporal mapping.<sup>103</sup> These devices can help address the need for point-of-care testing at the community level, where there is underreporting.

## CONCLUSIONS AND OUTLOOK

The availability of established diagnostic technologies (phase 3, Figure 3) have enabled researchers to plug-and-play in the design of COVID-19 diagnostics. Such technologies took decades to optimize, but they are now playing an important role in identifying and managing the spread of COVID-19. Lessons learned from the 2002 SARS outbreak have guided the development of COVID-19 identification and detection. Transmission electron microscopy was used to identify the morphology of the virus, genome sequencing was used to confirm the identity of the virus, and sequence data were used to help design PCR primers and probes. SARS-CoV took 5 months to be identified. The same techniques were used to

identify SARS-CoV-2 in only 3 weeks.<sup>104</sup> The rapid identification and sequencing of SARS-CoV-2 has enabled the rapid development of nucleic acid tests. These approaches provide a first line of defense against an outbreak. The next step being worked on is to establish serological tests because they are easier to administer and may complement nucleic acid tests for diagnosing COVID-19 infection. There is now a call for development of point-of-care tests and multiplex assays. Technologies that are straddling phases 2 and 3 (Figure 3) such as isothermal amplification, barcoding, and microfluidic technologies should be further developed so that they can become plug-and-play systems and can be rapidly implemented in an outbreak situation. The combination of diagnostics and smartphones should provide greater communication and surveillance. In conclusion, diagnostics are an important part of the toolbox for dealing with outbreaks because they enable healthcare workers to direct resources and efforts to patients with COVID-19. This process can curb the spread of infectious pathogens and reduce mortality.

Of note, data on COVID-19 are evolving quickly. Some of the specifics in this review may change as more studies become available. Many highlighted studies also identified weaknesses with their experimental study and design. Some referenced manuscripts are preprints and have not been peer-reviewed.

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<https://pubs.acs.org/10.1021/acsnano.0c02624>

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### Notes

The authors declare the following competing financial interest(s): Co-founded companies Luna Nanotech and Vigilant.

## ACKNOWLEDGMENTS

W.C.W.C., S.M., and J.B.G. acknowledge the Canadian Institutes of Health Research and Natural Sciences and Engineering Research Council of Canada through the Collaborative Health Research Program (CPG-158269 and 2015-06397). W.C.W.C. acknowledges the Canadian Research Chairs Program (950-223824). We also acknowledge the Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship (B.U.), the Barbara and Frank Milligan Graduate Fellowship (B.U., H.N.K., and P.K.), the Paul Cadario Doctoral Fellowship in Global Engineering (B.U., M.O., and H.N.K.), McCuaig-Throop Bursary for support (B.U.), Canadian Institutes of Health Research Vanier Scholarship (H.N.K.), the University of Toronto MD/Ph.D. program (H.N.K.), and National Research Council - Center for Research and Applications in Fluidic Technologies Fellowship (A. M.).

## GLOSSARY

**Angiotensin converting enzyme 2 (ACE2) receptor**, the cell receptor likely responsible for SARS-CoV-2 viral entry into cells; **COVID-19**, Coronavirus disease 2019; **bronchoalveolar lavage (BAL) fluid**, fluid collected using a bronchoscope (*i.e.*, procedure that looks at lungs and air passage) that is used to diagnose a lung infection; **computed tomography (CT)**, a non-invasive form of medical imaging that compiles cross-sectional images of the body; **Coronaviridae family**, family of enveloped viruses with positive-sense single-stranded RNA; **enzyme-linked immunosorbent assay (ELISA)**, a laboratory technique that quantifies proteins, peptides, antibodies, or hormones from the biological system; **isothermal amplification**, genetic multiplication techniques that occur at a single temperature; **lateral flow assays**, rapid, paper-based platforms that detect analytes at the point-of-care (most commonly of antigens and antibodies); **loop-mediated isothermal amplification (LAMP)**, an isothermal amplification technique commonly used for point-of-care testing. LAMP involves 4–6 primers and exponential amplification (genetic multiplication) of target DNA that produces concatenated DNA structures for detection; **microfluidics**, technologies that manipulate and control fluids at the microscale ( $10^{-6}$  m) or smaller; **multiplexing**, simultaneous testing of multiple target

molecules in a single sample; **nasopharyngeal swabs**, an elongated swab that collects secretions from the back of the patient's nose; **oropharyngeal swabs**, a swab that collects secretions from the patient's throat; **point-of-care testing**, diagnostic testing performed at or near the site of the patient; **reverse transcription polymerase chain reaction (RT-PCR)**, a nucleic acid amplification technique where RNA is converted into DNA and repeatedly multiplied for detection; **severe acquired respiratory syndrome coronavirus 2 (SARS-CoV-2)**, the name of the infective virus causing the COVID-19 disease; **serological testing**, diagnostic testing that measures the level of antibodies in blood; **sputum**, a mixture of saliva and mucus from the respiratory tract of a patient; **surveillance**, the collection and analysis of data for the prevention and control of a disease; **tracheal aspirates**, tracheal secretions collected for culturing and pathogen detection

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