Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 4

Testing and diagnosis of SARS-CoV-2 infection

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Chapter outline

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4.1 Introduction

The novel SARS-CoV-2 virus recently emerged in Wuhan, China, in December 2019, causing a new public health crisis threatening the world [1]. A group of patients with fever, shortness of breath, cough, and other symptoms were hospitalized [2]. Patients were scanned via computed tomography (CT), which showed a variety of turbidity (denser, confluent, and more profuse) compared to images of healthy lungs [3]. This finding led to the initial diagnosis of pneumonia [4]. Additional analysis of nucleic acid using real-time polymerase chain reaction (RT-PCR) of known pathogenic panels led to negative results, indicating that the cause of pneumonia was of unknown origin [1]. By January 2020, bronchoalveolar (BAL) fluid samples of patients were examined to detect a pathogen with a genetic sequence similar to that of the β-coronavirus B virus lineage.

The new pathogen was found to resemble 80%, 50%, and 96% of the genome of the acute respiratory syndrome (SARS-CoV) virus, the Middle East respiratory syndrome (MERS-CoV) virus, and bat coronavirus RaTG13, respectively [1,5]. The novel coronavirus was named SARS-CoV-2, the pathogen causing coronavirus disease 2019 (COVID-19) [4].

In the last 20 years, humans have faced three different outbreaks of the virus: SARS-CoV-1 in 2003, MERS-CoV in 2012, and the SARS-CoV-2 epidemic in 2019 [6]. Regardless of the fundamental nature of these three outbreaks of coronavirus, the most reasonable and sensible approaches to prevent and reduce the negative consequences of viral epidemics on humans require effective monitoring programs, along with laboratory preparation [7]. In the case of serious biologic hazards such as viral outbreaks, diagnostic laboratories play an important role in the rapid and accurate diagnosis and isolation of new microorganisms using the cornerstone in diagnostic virology, which are molecular diagnostic techniques [8,9]. In addition, the introduction of rapid molecular diagnostic and serological assessment techniques in reference diagnostic laboratories would enable rapid identification, isolation, and rapid treatment of COVID-19 cases. This demonstrates, once again, that laboratory medicine is inseparable to most care pathways [10] and may remain so for many years to come [7].

In this chapter, emphasis was laid on case definitions, specimen collection, existing molecular tests, and serological diagnostic tests, based on laboratory and point-of-care testing technology (POCT), used to diagnose COVID-19. In addition, it will summarize the associated vulnerabilities and gaps in the performance of the current diagnostic technologies that are likely to have serious consequences against the global efforts to contain the outbreak.

4.2 Case definitions

The World Health Organization (WHO) regularly updates the global surveillance for human infection with COVID-19 document which includes case definitions [11]. This information may be revised later based on the confirmed

different cases of incidence, the results of epidemiological finding, and the level of epidemics. The suspected case definition and probable case definition can be changed according to newly updated information from the WHO or other accepted guidelines. For easy reference, case definitions are included below.

4.2.1 Confirmed case

A person with laboratory confirmation of SARS-CoV-2 infection, irrespective of clinical signs and sympstoms (diagnostic tests: real-time RT-PCR, SARS-CoV-2 virus isolation).

4.2.2 Suspect case

A patient with acute respiratory illness (fever and at least one symptom/sign of respiratory disease, e.g., shortness of breath, cough), AND a travel history to or residence in a location reporting community transmission of COVID-19 during the 14 days prior to symptom onset; or

- A patient with any acute respiratory illness AND having been in contact with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset; or
- A patient with severe acute respiratory illness (fever and at least one symptom/sign of respiratory disease, e.g., shortness of breath, cough; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

4.2.3 Probable case

A suspect case for whom testing for the COVID-19 virus is inconclusive (inconclusive being the result of the test reported by the laboratory); or

- A suspect case for whom testing could not be performed for any reason.

4.3 Specimen type and priority

4.3.1 Specimen collection for molecular testing

The following are the acceptable upper respiratory specimens for diagnosis:

4.3.1.1 Upper respiratory specimens

All testing for SARS-CoV-2 should be performed in consultation with a healthcare provider. Specimens should be collected as soon as possible once a decision has been made to pursue testing, regardless of the time of symptom onset. The following tips address specimens' collection options.

For early diagnostic tests for SARS-CoV-2, the Centers for Disease Control and Prevention (CDC) recommends collecting and testing a specimen of upper respiratory. The following are acceptable specimens:

- **Oropharyngeal (OP)** specimen collected by a healthcare provider; or
- **Nasopharyngeal (NP)** specimen collected by a healthcare provider; or
- **Nasal mid-turbinate swab** collected by a healthcare provider or by a supervised onsite self-collection (using a flocked tapered swab); or
- **Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW)** specimen collected by a healthcare provider; or
- **Anterior nares (nasal swab) specimen** collected by a healthcare provider or by onsite or home self-collection (using a flocked or spun polyester swab).

Swabs should be placed immediately into a sterile transport tube containing 2−3 mL of either VTM (viral transport medium), sterile saline, or other accepted transport medium, unless employing a test designed to analyze a specimen directly, without placement in VTM, like some point-of-care tests.

The NW specimen and the nonbacteriostatic saline used to collect the specimen shall be immediately placed in a sterile transport container. Swab specimens should only be collected through swabs with an artificial tip (e.g., nylon or Dacron) and a plastic or aluminum shaft. Calcium alginate swabs are unacceptable and swabs with cotton tips and wooden shafts are not recommended.

After collecting the specimen, the person in charge of sampling should cut the swab using scissors so that it can fit inside the transport media tube. Once the swab has been cut, the scissors should be disinfected to ensure that there is no cross-contamination from one sample to the next.

4.3.1.2 Lower respiratory specimens

Examination of the lower respiratory tract specimen is also an option. For patients with a productive cough, sputum should be tested for SARS-CoV-2. Induction of sputum is not recommended. When under certain clinical conditions (e.g., patients under invasive mechanical ventilation), a BAL or an aspirate sample of the lower respiratory tract should be collected and determined as specimens of the lower respiratory tract.

- **Sputum**—Ask the patients to rinse their mouths with water and then take expectorate deep cough directly into a sterile, screw-cap sputum collection cup or dry sterile container.
- **BAL**—Collect 2−3 mL BAL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

4.3.2 Specimen collection for serology (IgG) testing

Collect at least 5 mL of blood in a plastic serum separator tube (SST). Tubes with gel serum separator are preferred. Centrifuge the SST according to blood

collection manufacturer's instructions for separation of serum from whole blood. SST should be put at 2−8°C after centrifuging the specimen. The tubes do not be freeze as it will result in hemolysis which is not recommended for testing.

4.4 Specimen storage

All specimens collected for molecular testing must be refrigerated (2−8°C) promptly after collection and couriered/shipped on cold packs within 72 h. Specimens being held for >72 h must be stored at −70°C and couriered/shipped on dry ice.

All specimens collected for serology testing must be refrigerated at (2−8°C) promptly after collection and couriered/shipped on cold packs within 5 days of collection. Specimens being held for >5 days must be stored at −20°C and couriered/shipped on dry ice.

4.5 Collecting and handling specimens safely

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to false negative test results.

For providers collecting specimens or within 6 feet of patients suspected to be infected with SARS-CoV-2, proper infection control and recommended personal protective equipment should be maintained, which includes an N95 or higher-level respirator (or facemask if a respirator is not available), gloves, eye protection, and a gown, when collecting specimens.

For providers who are handling specimens, but are not directly involved in collection (e.g., self-collection) and not working within 6 feet of the patient, standard precautions should be followed; gloves are recommended. Healthcare providers are recommended to wear a form of source control (cloth face covering or facemask) at all times while in the healthcare facility.

Personal protective equipment use can be minimized through patient self-collection while the healthcare personal maintains at least 6 feet of distance [12].

4.6 Diagnosis methods

Many diagnostic tests for SARS-CoV-2 are available so far, with more gaining emergency approval every day. These tests are largely based on four different techniques: (1) diagnostic chest imaging, (2) ribonucleic acid−based methods, (3) antibody-based methods, and (4) biosensors.

4.6.1 Diagnostic chest imaging

4.6.1.1 Chest radiography

Portable chest radiography (CXR) has the advantage of eliminating the need for patient transfer and may reduce the use of personal protective equipment.

However, CXR is insensitive to the diagnosis of primary disease, but can be used as a basis for follow-up imaging for disease progression. Wong et al. [13] and Guan et al. [14] in CXR showed a sensitivity of 59% for the initial diagnosis of COVID-19 abnormalities. Radiographic abnormalities, if present, are a mirror of chest CT scans, with bilateral lower zone- and peripherally predominant consolidation and hazy opacities.

4.6.1.2 Computed tomography

Chest CT scans are noninvasive and involve taking many X-ray measurements at different angles across a patient's chest to produce cross-sectional images [15,16]. The images are analyzed by radiologists to evaluate for abnormal features that may cause a diagnosis [16]. The imaging features of COVID-19 are diverse and depend upon the infection stage after the onset of signs. In February 2020, Chinese studies showed that chest CT achieved a better sensitivity for the diagnosis of COVID-19 compared with initial RT-PCR tests of pharyngeal samples [3,17]. Finally, the National Health Commission of China briefly accepted chest CT findings of viral infection as diagnostic of SARS-CoV-2 infection [18,19]. The typical appearance of COVID-19 on chest CT consists of bilateral and peripheral ground-glass opacities (areas of hazy opacity) [20] and consolidations of the lungs (fluid or solid material in compressible lung tissue) [21,22]. However, such findings are nonspecific; the differential diagnosis includes organizing pneumonia and other infections [23], drug reactions, and other inflammatory processes. Consequently, using CT to screen for COVID-19 may result in false positives. Moreover, the presence of abnormalities not typically associated with SARS-CoV-2 infection, including pure consolidation, cavitation, thoracic lymphadenopathy, and nodules, suggests a different etiology [19,21]. On the other hand, De Wever et al. revealed that ground-glass opacities are most prominent 0−4 days after onset of symptoms. As SARS-CoV-2 infection progresses, in addition to ground-glass opacities, crazy-paving patterns develop [22], followed by increasing the lungs consolidation [21,22].

The main content of using CT for COVID-19 is that it has low specificity (25%) because the imaging properties overlap with other viral pneumonias [3]. Based on these imaging features, several retrospective studies have shown that CT scans have a higher sensitivity (86%−98%) and have improved the false negative rate compared to RT-PCR [3,14,17,24].

4.6.1.3 Lung ultrasound

Lung ultrasound suggests low-cost, POCT assessment of the lung parenchyma without ionizing radiation. The modality is especially applicable in resource-limited settings [25]. The scholars showed that sonographic results in COVID-19 patients associated with typical CT abnormalities [26]. The predominantly peripheral distribution of lung involvement facilitates sonographic visibility.

Characteristic findings include irregular pleural lines and thickened B lines (edema) and the eventual appearance of A lines (air) during recovery. Peng et al. suggest that ultrasound may be applicable to guide prone positioning and monitor recruitment maneuvers [26].

4.6.2 Ribonucleic acid–based methods

4.6.2.1 RT-qPCR

Polymerase chain reaction (PCR) is a process that causes a very small well-defined segment of DNA to be amplified, or multiplied many hundreds of thousands of times, so there is enough of it to be detected and analyzed. Viruses like SARS-CoV-2 do not contain DNA but they do have only RNA. Reverse transcription polymerase chain reaction (RT-PCR) is a method that first uses reverse transcription to change the extracted RNA into DNA and then uses PCR to amplify a piece of the resulting DNA, producing enough to be tested in order to determine if it matches the genetic code of SARS-CoV-2 [27–29]. Altogether, the combined method has been described as real-time RT-PCR [30] or quantitative RT-PCR [31] and is sometimes abbreviated qRT-PCR [32] or rRT-PCR [33] or RT-qPCR [34], although sometimes just RT-PCR or PCR is used as an abbreviation.

Quantitative RT-qPCR is routinely used to detect the viruses, and following SARS-CoV-2 initial characterization, Chinese and American Centers for Disease Control and Prevention and other relevant departments worldwide rapidly employed molecular assays for detection of SARS-CoV-2 in clinical samples [35–37], which has high sensitivity, rapid detection, and other desirable characteristics.

Because of its high sensitivity, simplicity, and high sequence specificity, PCR-based methods are routinely and reliably capable of evaluating in patients with coronavirus infection [27,38,39]. RT-PCR is significantly more sensitive than conventional methods [40,41] and is routinely employed as the predominant method to detection most coronaviruses [42,43], including SARS-CoV-2 [44].

These, and approaches by other researchers, are mostly employed development of RT-PCR methods to detect SARS-CoV-2, predominantly targeting different combinations of the envelope (*E*), nucleocapsid (*N*), open reading frame (*ORF*), and RNA-dependent RNA polymerase (*RdRp*) genes (Fig. 4.1) [1,35–37,46,47].

The inconsistency of RT-qPCR can be related to many various factors, including the diversity that occurs in viral RNA sequences, which subsequently affects findings that use primers in the *N* and *ORF1a/b* genes.

The influence of variation in viral RNA sequences can be minimized by the mismatch-tolerant amplification methods [48,49] which would be very helpful for improving the sensitivity and reliability of RNA detection.

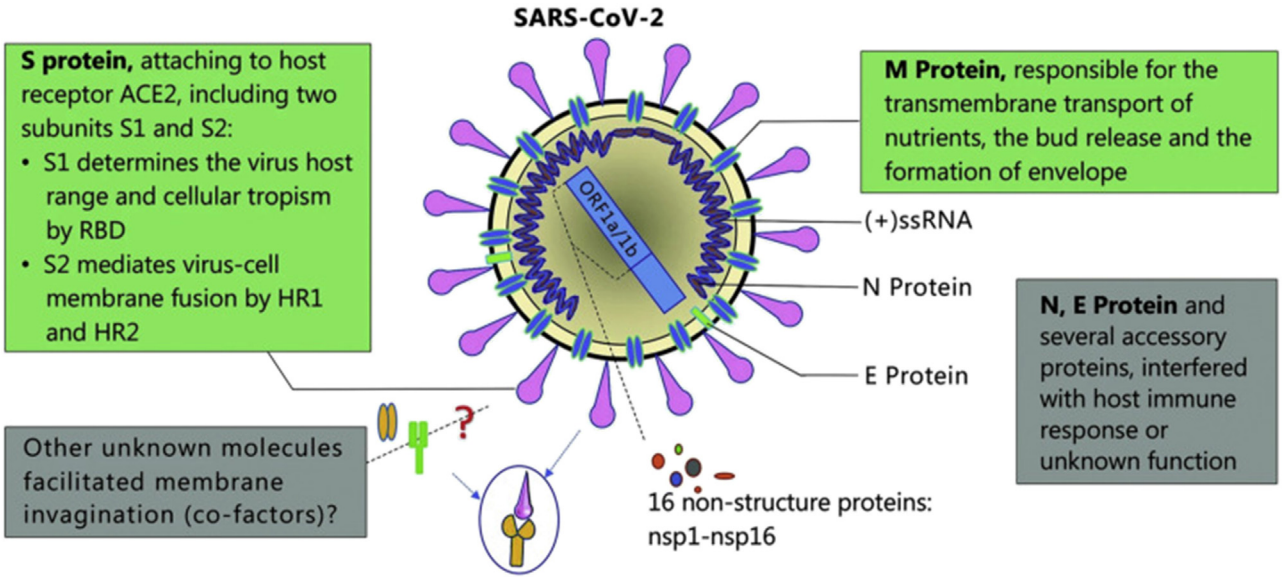

FIGURE 4.1 Schematic diagram of the SARS-CoV-2 structure. Structural proteins, including spike (S) glycoprotein, nucleocapsid (N) protein, matrix (M) protein, small envelope (E) protein, and also several accessory proteins. *Modified from Ref. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Milit Med Res March 13, 2020;7(1):11. PubMed PMID: 32169119. Pubmed Central PMCID: PMC7068984. Epub 2020/03/15. eng.*

Another factor that thwarts the accuracy and consistency of RT-qPCR tests is sampling procedures, since the viral loads vary in different anatomic sites [50].

In the recent months, many scientific teams and companies have successively developed methods to detect SARS-CoV-2 [47,51,52], but different methods have different detection efficiencies and some produce more false-negatives [53,54]. Therefore, improving the detection efficiency is one of the most important tasks at present.

A one-step RT-qPCR targeting *ORF1b* or nucleocapsid (*N*) gene of SARS-CoV-2 can detect 10 copies/reaction of plasmid DNA or 2×10^{-4}–2000 $TCID_{50}$ (50% tissue culture infective doses)/reaction of RNA extracted from virus cultures [47]. However, this method was designed to react with SARS-CoV-2 and its closely related viruses, such as SARS coronavirus [47], which may lead to false-positive reactions for SARS-CoV-2 identification.

Furthermore, Chan and colleagues developed a novel RT-qPCR assay targeting the RNA-dependent RNA polymerase (RdRp)/helicase (Hel) of SARS-CoV-2 and found that the limit of detection (LOD) of the assay was 1.8 $TCID_{50}$/ml with genomic RNA and 11.2 RNA copies/reaction with in vitro RNA transcripts, which has higher analytical sensitivity than the widely used RdRp-P2 assay [55].

Notably, the COVID-19-RdRp/Hel assay did not cross-react with other human origin coronaviruses and respiratory pathogens [55], which can be used to differentiate SARS-CoV-2 and other respiratory pathogens.

A deal, however, especially in the current demanding times, is that such analysis needs different specialist and expensive items of equipment, alongside highly professional analysts. Moreover, current PCR-based methods of analysis need upwards of 4–8 h to process.

Indeed, improved methods of quantitative RT-PCR characterized by rapid detection, high sensitivity, and specificity are often prescribed as a gold standard for virus detection [27]. However, further novel PCR-based methods also present enhanced specificity and assay sensitivity.

4.6.2.2 Reverse transcription loop-mediated isothermal amplification

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a novel isothermal nucleic acid amplification method, and to overcome the impediments of RT-qPCR's time-consuming and costliness but still be able to detect nucleic acid of pathogens, RT-LAMP–based methods are developed [56,57]. LAMP assays are meaningfully rapid and do not need expensive instruments or reagents, which helps in cost decrease for coronavirus detection [27].

The LAMP exhibits increased sensitivity and specificity due to an exponential amplification feature that utilizes six different target sequences simultaneously identified by separate distinct primers in the same reaction under a constant temperature of 60–65°C [58–60].

The RT-LAMP is performed in one step at isothermal conditions, and the results are obtained within 15−40 min, by targeting the *ORF1a/b*, *S*, *E*, and/or *N* gene of SARS-CoV-2 [61−63].

The assay can detect the virus in the throat and nasal swabs, with an LOD in the sample of about 5−10 RNA copies and 99%−100% agreement with the commercial RT-qPCR [63,64].

Numerous [28,65] studies have now shown the successful application of RT-LAMP assays in various forms to detect coronavirus RNA in patients' samples [66−69] demonstrating that 1−10 copies of viral RNA template per reaction were sufficient for successful detection, which were ∼100-fold more sensitive than conventional RT-PCR methods [68−72].

Moreover, unpurified samples could be evaluated directly using LAMP [73]. This reveals that high-throughput examination is possible when using unpurified specimens mixed with noninstrumental (e.g., colorimetric) evaluation [63]. Yu et al. [57] invented an isothermal LAMP-based approach for rapid colorimetric evaluation of SARS-CoV-2. The sensitivity was 97.6% (42/43) and readout time was within 30 min.

El-Tholoth et al. [74] recently described the design of a two-stage LAMP strategy, which could be carried out in closed tubes with either colorimetric or fluorescence detection. Performance of such determinations was not only comparable with conventional RT-PCR assays, but also exhibited ∼10 times higher sensitivity when testing purified targets. Similarly, Lamb et al. [75] also explained successful and rapid detection of SARS-CoV-2 RNA within 30 min of experimentation. However, with significant advances, these methods and assays have not yet been applied to confirmed patient samples, with both these studies relying upon "simulated" patient samples where blood and swabs samples were artificially "spiked" with RNA of SARS-CoV-2 [76].

Recent studies showed that an RT-LAMP targeting the *N* gene of SARS-CoV-2 can specifically measure viral RNAs of SARS-CoV-2 but has no cross-reactivity with other coronaviruses, as well as other respiratory disease−causing viruses and human infectious influenza viruses [77]. These results reveal that the RT-LAMP method has a wider commercial application for SARS-CoV-2 diagnosis due to its relatively simple operation and low technical requirements for operators. Kashir and Yaqinuddin [76] hypothesized that LAMP assay will be a rapid, cost-effective, and simple method that could be applied within the field at short notice and utilized by users with even limited training. All the equipment needed would be a hot block or heater capable of differential heating. Reagent-wise, the costs would be similar to that of RT-PCR, but the real advantage of this would be the speed of this assay, yielding results within an hour of testing, compared to 4−8 h taken with RT-PCR methods. The aim is not necessarily a quantitative measure of infection, but rather a simple negative/positive assay for quick detection/confirmation. They consider that this strategy should be applied rapidly and confirmed for viability with clinical samples, before being rolled out for mass diagnostic

testing in current times. As the growing number of suspected SARS-CoV-2 cases increases the capacity of many hospitals, many patients remain untested impeding efforts to the disease control. A rapid POCT for the COVID-19 is urgently needed, which professionals suggest to be the LAMP method of detection [76]. Of course, however, as with any emerging approach, there are some disadvantages associated with LAMP assays.

Such methodology prevents inclusion of an internal PCR inhibition control, necessitating duplication of reactions while testing. Another disadvantage of the perceived complexity of this method is the need for a complex primer design system that can limit the choice of target site and specificity or resolution. Moreover, as the end product is a big fragment, downstream applications like cloning are limited. Besides LAMP, other isothermal amplification approaches including recombinase polymerase amplification, multiple displacement amplification, rolling circle amplification, nucleic acid sequence-based amplification, and helicase-dependent amplification could be used for POCT-based nucleic acid evaluation [78] (Table 4.1).

4.6.2.3 Clustered regularly interspaced short palindromic repeats–based methods

Clustered regularly interspaced short palindromic repeats (CRISPR)–based diagnostic platforms have also been developed for POCT nucleic acid detection [95–98]. The CRISPR-based nucleic acid detection platforms combine recombinase polymerase amplification with CRISPR-based assay for specific recognition of targeted DNA or RNA sequences [80,99]. CRISPR-based RNA evaluation can achieve an attomolar (10^{-18}) level within 30 min [100]. An accurate and low-cost and CRISPR-Cas12–based lateral flow assay for detection of SARS-CoV-2 was published [101]. It can sensitively detect as low as 10 copies/μL for synthetic RNA or in vitro viral RNA transcripts. The results of the CRISPR-based methods can be analyzed by fluorescent or lateral flow strip in less than 1 h with a setup time of less than 15 min [80]. This method can be used in areas at greatest risk of transmitting SARS-CoV-2 infection, including airports, emergency departments, and local community hospitals, particularly in low-resource countries [102]. Furthermore, scientists compared the CRISPR-based methods and the RT-qPCR recommended by CDC/WHO for SARS-CoV-2 detection and found that RT-qPCR is more sensitive than the CRISPR-based assay, while the latter is more convenient and time-saving than RT-qPCR. However, due to the lack of clinical detection samples, the sensitivity and specificity of the CRISPR-based methods need further verification in the clinic [101]. The entire time of this assay is 45 min; in contrast, the RT-qPCR needs 4 h. Ding et al. designed all-in-one dual CRISPR-based assay for evaluation of SARS-CoV-2; the LOD was as low as 1.2 copies/mL [103]. Advantages over the current standard PCR-based technique for detecting SARS-CoV-2 include increased speed and the fact that the

TABLE 4.1 Emerging diagnostics being developed for SARS-CoV-2 based on nucleic acid and protein.

Platform	Biomarker	POCT (Y/N)	Type of technology	How it works	Types of clinical sample	Clinical sample tested
CRISPR [79]	Nucleic acid	Y	RPA	PCR, perform CRISPR/Ca9-mediated lateral flow nucleic assay (CASLFA)	Serum	110
CRISPR [80]	Nucleic acid	Y	RT-RPA	RPA, SHERLOCK multiplexed signal detection using fluorescence	Nasopharyngeal swabs	384
LAMP [81]	Nucleic acid	N	LAMP	Isothermal DNA synthesis via self-recurring strand displacement reactions; positive detection leads to elevated sample turbidity	Throat swabs	53
RPA [82]	Nucleic acid	N	RPA	Forward and reverse primers blind to DNA and amplify strands at 37°C	Fecal and nasal swabs	30
NASBA [83]	Nucleic acid	N	Real-time NASBA	Transcription-based amplification for RNA targets	Nasal swabs	138
RCA [84]	Nucleic acid	N	Rolling circle amplification	DNA polymerase used to extend a circular primer and repeatedly replicate the sequence	Serum	7
RT-LAMP [85]	Nucleic acid	N	LAMP	Reverse transcriptase LAMP reaction for RNA targets	Nasopharyngeal aspirates	59
Smartphone dongle [86]	Protein	Y	ELISA	Microfluidics-based cassette operating an ELISA	Blood	96
Quantum dot barcode [87]	Nucleic acid	Y	Barcode	Multiplexed quantum beads capture viral DNA for RPA detection	Serum	72

Magnetic bead [88]	Nucleic acid	N	Magnetic	Magnetic beads isolate bacteria for PCR detection	Stool	17
Paramagnetic bead [89]	Protein	N	Magnetic biosensor	Magnetic separation of protein targets	Serum	12
Magnetic bead isolation [90]	Whole bacteria	N	Magnetic separation	Magnetic isolation of bacteria	Synovia	12
ELISA [91]	Protein	N	ELISA	Enzymatic reaction to produce colored product in presence of target	Serum	30
SIMOA [92]	Protein	N	Digital ELISA	Digital readout of colored product by enzymatic reaction in presence of target	Serum	30
Bio-barcode assay [93]	Protein	N	DNA-assisted immunoassay	Protein signal is indirectly detected by amplifying DNA conjugated to gold nanoparticle	Serum	18
Rapid antigen test [94]	Protein	Y	Lateral flow	Gold-coated antibodies produce colorimetric signal on paper in presence of target	Serum	117

CRISPR, clustered regularly interspaced short palindromic repeats; *ELISA*, enzyme-linked immunosorbent assay; *LAMP*, loop-mediated isothermal amplification; *NASBA*, nucleic acid sequence-based amplification; *POCT*, point-of-care testing; *RCA*, rolling circle amplification; *RPA*, recombinase polymerase amplification; *RT-LAMP*, reverse transcription loop-mediated isothermal amplification; *SIMOA*, single molecule array.

Reprint from Ref. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: the disease and tools for detection. ACS Nano 2020;14(4):3822–35. PubMed PMID: 32223179. Epub 03/30. eng.

tests use commercially available "off-the-shelf" reagents without the need for expensive laboratory equipment. The fact that there is no need for any specialized laboratory equipment to obtain a result further raises the exciting possibility that such CRISPR-based assays could eventually be released as kits that could be used at home. As testing continues to be at the heart of any successful coronavirus containment strategy, the development of faster and simplified ways of obtaining a diagnosis holds great promise in fight against SARS-CoV-2.

4.6.2.4 Digital PCR

Digital PCR (dPCR) is a PCR technique known for its higher sensitivity and precision over classical PCR [104,105]. Digital PCR uses similar assay substances as used in standard analog determinations, but counts the total number of individual target molecules in a digital format, enabling many applications that have restricted sample availability and require high sensitivity. Digital PCR determinations are carried out by dividing the sample and qPCR test mixture into a very large number of separate small volume reactions, such that there is either zero or one target molecule present in any individual reaction [106–108]. This is the basic notion for making "digital" detections. Digital PCR has also been shown to be more resistant to PCR inhibitors [109]. Recent studies have confirmed high sensitivity of RT-dPCR for the detection of SARS-CoV-2 [110,111]. Digital PCR method can meaningfully improve the accuracy and sensitivity of COVID-19 diagnosis. The LOD of the optimized dPCR is at least 10 times lower than that of RT-qPCR. The overall specificity, sensitivity, and accuracy of RT-dPCR protocol for RNA detection were 100%, 90%, and 93%, respectively [112].

4.6.3 Antibody-based methods

Although nucleotide acid–based approaches are the recommended methods by WHO and many groups, some professionals have recently showed that the sensitivity of nucleic acid evaluation in SARS-CoV-2 is low, even as low as 42.10% [3,53,113], and there are several limitations in nucleotide acid–based detections, such as long turnaround time, complex operation, expensive equipment, and trained technicians [114].

Furthermore, in some cases of nucleotide acid–based detection, a positive result will find after repeated negative tests. Throat or nasopharyngeal swabs are negative many times, but finally, positive results are diagnosed in sputum specimens or respiratory BAL fluid samples [53,113,114]. Therefore, many experts suggest using specific antibody detection as a supplement for nucleic acid detection, because the antibody-based methods are relatively cheap, easy to operate, and have low technical requirements [115]. The antigens and antibodies of viral protein that are exposed to a SARS-CoV-2 infection can be

used for COVID-19 diagnosing. Changes in viral load over the course of the infection may make viral proteins difficult to evaluate. For example, Lung et al. [80] revealed high salivary viral loads in the first week after symptoms onset, which gradually reduced with time. In contrast, antibodies produced in response to viral proteins may provide a larger window of time for indirect SARS-CoV-2 detecting. Antibody tests can be especially beneficial for surveillance of SARS-CoV-2. Serological antibody test is important for symptomatic patients who are negative in RT-qPCR assays. Immunoglobulin M (IgM) tends to show a recent exposure to SARS-CoV-2, whereas detection of SARS-CoV-2–related IgG reveals virus exposure some time ago. One potential problem with developing accurate serological tests includes potential cross-reactivity of SARS-CoV-2 antibodies with antibodies produced against other family members of coronaviruses. Lv et al. measured plasma samples from SARS-CoV-2 patients against the S protein of SARS-CoV-2 and SARS-CoV and found a high frequency of cross-reactivity [116]. Nowadays, serological tests including blood tests for specific antibodies are in development [117]. Zhang et al. detected IgG and IgM from human serum of SARS-CoV-2 patients using an enzyme-linked immunoassay (ELISA) method [117]. They tested SARS-CoV-2–positive patient samples (confirmed by RT-PCR) and saw the levels of these antibodies elevated over the first 5 days after the onset of symptom. On day zero, 81% and 50% of patients were positive for IgG and IgM, respectively, but this increased to 100% and 81% at day five [117]. Recombinant SARS-CoV-2 nucleocapsid protein (rN) and spike protein (rS) are determined via antigens in ELISA for COVID-19 IgG/IgM detection. The results show that the rS-based ELISA has a meaningfully higher sensitivity than that of the rN-based ELISA [118]. As reported, antibody-based methods targeting IgG and IgM induced by the recombinant N and S proteins of SARS-CoV-2 are consistent with the results obtained by nucleic acid–based assay [114,119,120]. Furthermore, the receptor-binding domain (RBD) of the S protein revealed a better antigenicity than that of the N protein for the testing of SARS-CoV-2 infection. Moreover, a new report reported that IgA level in patient serum is positively correlated with the severity of the SARS-CoV-2 [121], indicating that serum IgA can also be used as a biological marker for the COVID-19 identification. It was published that ELISA is superior to lateral flow immunoassay in quantification and specific detection of SARS-CoV-2 IgG and IgM and is highly sensitive to IgG 10 days after symptoms onset.

Lateral flow immunochromatographic strip (LFICS) has been developed and approved for diagnosis of SARS-CoV-2 [121]. It can be a POCT disease diagnostic tool because it is portable, inexpensive, and without requiring power. The LFICS includes a conjugate pad (CP), sample pad (SP), wick/absorbent pad, and nitrocellulose membrane (NC) and works similar to a pregnancy test. Gold nanoparticles (Au NPs) colloid-based LFICS, which is also known as colloidal gold immunochromatographic assay (CGICA), can

measure IgG and IgM antibodies simultaneously against SARS-CoV-2 virus in blood up to 15 min. A study conducted by Li et al. shows that the overall testing sensitivity of LFICS assay is 88.66% and the specificity is 90.63% [114]. Xiang et al. compared the ELISA and CGICA for detection of IgM and IgG. It is demonstrated that there is no evident difference between ELISA and CGICA [122]. Recently, Lin et al. found that serological chemiluminescence immunoassay based on the recombinant N protein of SARS-CoV-2 had a larger performance for recognition of COVID-19 than that of the ELISA kit, with more reliable specificity and sensitivity of 97.5% and 82.28%, respectively [123]. Therefore, antibody-based methods are effective approaches to evaluate COVID-19. To improve the detection efficiency, several groups developed antibody-based methods for simultaneous detection of IgG and IgM [114,124] and found that the sensitivity of test detecting IgM and IgG simultaneously was significantly higher than the nucleic acid, IgM or IgG single detection [125]. Besides, the IgA/IgG or IgA/IgM/IgG combination can provide improved diagnostic reliability compared to conventional IgM/IgG combinations [126]. Clinically, specific IgA and IgM against SARS-CoV-2 can be detected 7 days after virus infection or 3−4 days after symptoms appear, and specific IgG of the virus appears in 7−10 days after SARS-CoV-2 infection [114,124,127]. IgG titers increased within 3 weeks after symptoms appeared and the median concentration reached a peak of 16.47 μg/mL in 21−25 days after onset and began to decrease at the eighth week, but remained above the detection threshold [128]. For patients of various stages, the sensitivities of GICA strips targeting viral IgM or IgG antibody were 11.1%, 92.9%, and 96.8% for the nucleic acid confirmed patients of the early (within first week after symptom onset), middle (within second week after symptom onset), and late stage of the COVID-19 (over 2 weeks), respectively [129]. These findings suggest that antibody evaluation of SARS-CoV-2 can be accomplished in the middle to later stages of COVID-19. Commonly, a clinical diagnosis can be terminated in as little as 5−15 min using antibody-based methods (particularly by commonly used serological GICA strip), via various types of blood samples, such as serum and plasma of venous blood or fingerstick blood [114,129]. Therefore, combined with nucleic acid detection, the detection of virus-specific antibody can significantly reduce “false-negatives” of SARS-CoV-2 infection at the clinical level. Table 4.1 shows emerging diagnostics being developed for SARS-CoV-2 based on nucleic acid and protein.

4.6.4 Biosensors

Although RNA detection based on chest imaging, RT-qPCR, and antibody detection has been developed, all of these methods have certain practical limitations. Biosensors, especially the smartphone-driven biosensors, have the potential to be alternative tools since they can provide fast, accurate, and

sensitive early detection [130−133]. These biosensors include electrochemical (EC) biosensors, colorimetric biosensor, fluorescence-based biosensor, surface-enhanced Raman scattering (SERS), localized surface plasmon resonance, piezoelectric microcantilever sensors, quartz crystal microbalance, etc. [134−136] Among them, label-free electrical/EC biosensors and SERS are the most popular [137,138]. Electrical/EC biosensors possess the advantages of low cost, simplicity, and are more easily miniaturized and mass fabricated. They can also be used as POCT devices at home or at the doctor's office [139,140]. SERS is known as an ultrasensitive molecular spectroscopy technique that has no interference from water, making it a distinct advantage in the identification of bio-samples. A SERS-based biosensor does not require extensive sample preparation steps and has high enough sensitivity to detect trace amounts of bioparticles, and under special circumstances, it can even be capable of single-molecule detection [141]. Most of the biomarkers of SARS-CoV-2 can be detected by biosensors [142]. Recombinant spike protein S1 was used as a biomarker for two different coronavirus detection by EC immunosensor [143]. The design of the electrode array enables the multiplexed measurement. The test can be completed within 20 min and the LOD was reached as 1.0 pg/mL for MERS-CoV and 0.4 pg/mL for human coronavirus. The EC immunosensor was also successfully used to nasal specimen. The EC biosensor can also be used to detect the virus nucleic acid. Researchers developed a cheap, simple, and easy-to-handle EC genosensor for the detection of SARS-CoV [65]. Field-effect transistor (FET)−based biosensor is one type of electrical biosensor. A graphene-based FET biosensor was reported to detect the SARS-CoV-2 and its spike protein in clinical samples [144]. The results demonstrated that the LOD of S protein was 1 fg/mL in phosphate-buffered saline and 100 fg/mL in clinical transport medium. The LOD of SARS-CoV-2 in culture medium was 1.6×10^1 pfu/mL and in clinical samples was 2.42×10^2 copies/mL. The biosensor could discriminate the SARS-CoV-2 S protein from that of MERS-CoV. The success of this biosensor confirmed the potential for SARS-CoV-2 diagnosis using antigen protein in the transport medium of NP swabs. It is also confirmed that the biosensor can detect the SARS-CoV-2 from clinical samples [142]. Two respiratory viruses, human adenovirus (HAdV) and influenza A H1N1 virus, were detected by the SERS-based biosensor [145]. The LOD for HAdV and H1N1 were 10 and 50 pfu/mL, respectively, which are 2000 times more sensitive than those from the standard colloidal gold strip method. Porcine circovirus type 2, porcine parvovirus pseudorabies, and porcine parvovirus virus were detected by SERS based on a porous carbon substrate decorated with silver nanoparticles [146]. The LOD of these three are as low as 1×10^7 copies m/L. The principal components analysis was used to discriminate the viruses based on the SERS spectra [142]. More recently, a plasmonic biosensor was reported to detected RNA of SARS-CoV-2 through nucleic acid hybridization [147]. The cDNA sequences were

fixed on the gold nanoislands (AuNIs) as receptors. Both localized surface plasmonic photothermal (PPT) and plasmon resonance effects were used collaboratively to increase the signal. The LOD for detection of the *RdRp* gene was about 0.22 p.m. With the in situ PPT enhancement on gold AuNIs chips, *RdRp* genes from SARS-CoV and SARS-CoV-2 can be accurately distinguished [142].

4.7 Future projection

Lessons learned from the 2002 SARS outbreak have guided the development of COVID-19 identification and detection. Transmission electron microscopy was used to detection the morphology of the virus, genome sequencing was used to confirm the similarity of the virus, and sequence data were used to help design of PCR primers and probes. SARS-CoV took 5 months to be recognized. The similar approaches were used to identify SARS-CoV-2 in only 3 weeks [148].

The availability of established diagnostic technologies has enabled scientists to plug and play in the design of SARS-CoV-2 diagnostics. Such technologies took decades to optimize, but they are now playing an important role in identifying and managing the outbreak of SARS-CoV-2 [4].

There is now an invitation for development of POCT and multiplex assays. Technologies such as isothermal amplification, barcoding, and microfluidic technologies should be further developed so that they can become plug-and-play systems and can be quickly used in an outbreak situation [4], similar to novel SARS-CoV-2 outbreak. The mix of diagnostics and new communication devices should provide higher communication and surveillance. At the same time, the establishment of a differentiation method between SARS-CoV-2 and other respiratory viruses is also urgently needed [115].

On note, nucleic acid—based methods are sensitive, but prone to false-positive. The sensitivity of the antibody-based method is slightly lower, but the accuracy is higher. Therefore, it is suggested that the two methods should be mixed to improve the detection accuracy of COVID-19 [115].

4.8 Conclusion

The rapid sequencing and identification of SARS-CoV-2 has enabled the rapid development of nucleic acid assays. These methods provide a first line of defense against an outbreak. The next step being worked on is to establish serological assays because they are easier to administer and may complement nucleic acid assays for diagnosing infection of COVID-19 [148].

Furthermore, making a risk-free sample preparation method for detection is one of the urgent tasks to be solved at present. Also, because a few recovered patients that have been discharged from hospitals have diagnosed positive in

nucleic acid tests, it is still necessary to develop new specific and sensitive detection approaches for the confirmation of virus-infected persons, recovered patients, and carriers [115].

In conclusion, diagnostics are an important part of the toolbox for dealing with outbreaks because they enable healthcare providers to direct resources and efforts to COVID-19 patients. This process can curb the spread of infectious pathogens and decrease mortality [4].

List of abbreviation

AuNPs Gold nanoparticles
AuNIs Gold nanoislands
BAL Bronchoalveolar
CDC Centers for Disease Control and Prevention
CGICA Colloidal gold immunochromatographic assay
CLIA Chemiluminescence immunoassay
COVID-19 Coronavirus disease 2019
CP Conjugate pad
CRISPR Clustered regularly interspaced short palindromic repeats
CT Computed tomography
CXR Chest radiography
dPCR Digital PCR
E Envelope
EC Electrochemical
ELISA Enzyme-linked immunosorbent assay
FET Field-effect transistor
HAD Helicase-dependent amplification
HAdV Human adenovirus
IgA Immunoglobulin A
IgG Immunoglobulin G
IgM Immunoglobulin M
LAMP Loop-mediated isothermal amplification
LFICS Lateral flow immunochromatographic strip
LOD Limit of detection
LSPR Localized surface plasmon resonance
MDA Multiple displacement amplification
MERS-CoV Middle East respiratory syndrome coronavirus
N Nucleocapsid
NASBA Nucleic acid sequence-based amplification
NC Nitrocellulose membrane
NP Nasopharyngeal
NW Nasopharyngeal wash/aspirate or nasal wash/aspirate
OP Oropharyngeal
ORF Open reading frame
PCR Polymerase chain reaction
PEMS Piezoelectric microcantilever sensors
POCT point-of-care testing

PPT Plasmonic photothermal
QCM Quartz crystal microbalance
Qpcr Quantitative polymerase chain reaction
RCA Rolling circle amplification
RdRp RNA-dependent RNA polymerase
RPA Recombinase polymerase amplification
RT-LAMP Reverse transcription loop-mediated isothermal amplification
RT-PCR Reverse transcription polymerase chain reaction
SARS-CoV Acute respiratory syndrome virus
SARS-CoV-2 Severe acute respiratory syndrome coronavirus 2
SERS Surface-enhanced Raman scattering
SIMOA Single molecule array
SP Sample pad
$TCID_{50}$ 50% Tissue culture infective doses
VTM Viral transport medium

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