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Molecular Diagnosis of Coronavirus Disease 2019

Claudia C. dos Santos, MD^{1,2}; Barbara A. Zehnbauer, PhD³; Uriel Trahtemberg, MD¹; John Marshall, MD^{1,4}

Objectives: To review molecular diagnostics for coronavirus disease 2019. The world is in the midst of a coronavirus disease 2019 pandemic. Containing the spread of the severe acute respiratory distress coronavirus is critical. Instrumental to the future success is the ability to reliably and reproducibly detect this inciting pathogen to inform public health containment policies and treatment decisions.

Data Sources: Molecular diagnostics focusing on molecular detection methodologies for detection of the virus and the presence of the disease.

Study Selection: Narrative review.

Data Extraction: Literature, PubMed, Scopus, and official government documents.

Data Synthesis: Diagnosing severe acute respiratory syndrome coronavirus is done through real-time reverse transcriptase-polymerase chain reaction tests, cell culture, and serology. For patients, diagnostics are an integral part of a full medical history, physical examinations, blood tests, and diagnostic imaging.

Conclusions: Here, we review current approaches to the molecular diagnosis of coronavirus disease 2019.

Key Words: coronavirus disease 2019; coronavirus disease 2019 serologic tests; nucleic acid amplification tests; real-time reverse transcription polymerase chain reaction diagnostic primer panel; severe acute respiratory syndrome coronavirus 2

The clinical diagnosis of suspected coronavirus disease 2019 (COVID-19) is mainly based on epidemiologic history, clinical manifestations, and auxiliary examinations, such as CT

¹Keenan Research Center for Biomedical Research, Unity Health Toronto, Toronto, ON, Canada.

²Interdepartmental Division of Critical Care and Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada.

³Department of Pathology, Emory University School of Medicine, Atlanta, GA.

⁴Department of Surgery, University of Toronto, Toronto, ON, Canada.

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Crit Care Expl 2020; 2:e0184

DOI: 10.1097/CCE.000000000000184

scan (1, 2). However, clinical symptoms and signs that a patient is infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the virus responsible for COVID-19—are nonspecific, including cough, fever, dyspnea, diarrhea, vomiting, abdominal pain, viral pneumonia, and respiratory distress (3–5). Radiological as well as pathologic findings are consistent with diffuse airspace disease, injury to the alveolar epithelial cells, hyaline membrane formation, inflammatory cell infiltration, and hyperplasia of type II pneumocytes, consistent with acute lung injury, but lacking in sensitivity and specificity (6), and showing important distinctions from acute respiratory distress syndrome (7) with implications for therapy (2). The spectrum of symptoms/disease caused by COVID-19 also grows every day including neurologic symptoms, venous thromboembolism, myocarditis, and other (see situation report [8]). Molecular diagnostics are critical for the diagnosis of COVID-19 in order to inform decisions related to infection control and treatment.

Molecular diagnostics include a variety of methods to analyze biological markers in affected individuals (9) that enable detection of (1) the inciting pathogen, that is, SARS-CoV-2 particles and/or components of SARS-CoV-2 in body fluids and/or tissues; (2) the presence of disease as demonstrated by evidence that the immune system has been exposed to viral antigens, that is, antibodies against components of SARS-CoV-2; and finally (3) diagnostics testing integrated into a full medical history, enduring physical examinations, blood tests, and diagnostic imaging including chest radiographs, chest CT, and MRI. Here, we will focus on reviewing key developments in the molecular diagnosis of COVID-19 (4, 9), both nucleic acid and protein based. A guideline for diagnostic testing for COVID-19 has recently been published and reviews current approaches in detail (10, 11).

TESTING GUIDELINES FOR PATIENTS WHO MEET THE SUSPECT CASE DEFINITION

During the pandemic, we have seen definitions for patient under investigation change as well as the recommendations for testing (common seen in Fig. 1) with healthcare institutions (and even whole geographical regions) either moving or moved toward universal testing (10, 12, 13). The guidelines for COVID-19 diagnosis are available from the Infectious Disease Society of America website (<https://www.idsociety.org/public-health/COVID-19-Resource-Center/>) (10). The U.S. Centers for Disease Control and Prevention

The CDC guide for who should be tested

Priority should be given to:

1. Hospitalized patients who have signs and symptoms compatible with COVID-19 in order to inform decisions related to infection control

<https://www.cdc.gov/coronavirus/2019-ncov/hcp/index.html>

2. Symptomatic individuals such as, older adults and individuals with chronic medical conditions and/or an immunocompromised state that may put them at higher risk for poor outcomes

3. Any persons including healthcare personnel, who within 14 days of symptom onset have had close contact* with a suspect or laboratory-confirmed COVID-19 patient, or who have a history of travel from affected geographic areas[#] within 14 days of their symptom onset.

(<https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-risk-assessment-hcp.html>)

* Close contact is defined as:

a) being within approximately 6 feet (2 meters) of a COVID19 case for a prolonged period of time; close contact can occur while caring, living with, visiting, or sharing a healthcare waiting area or room with a COVID-19 case

b) having direct contact with infectious secretions of a COVID-19 case. If such contact occurs while not wearing recommended personal protective equipment (PPE) (e.g., gowns, gloves, NIOSH-certified disposable N95 respirator, eye protection), criteria for PUI consideration are met.

[#]Affected areas :

Are defined as geographic regions where sustained community transmission has been identified. For a list of relevant affected areas, see CDC's Coronavirus Disease 2019 Information for Travel

Figure 1. Centers for Disease Control and Prevention (CDC) guide for who should be tested. COVID19 = coronavirus disease 2019, NIOSH = National Institute for Occupational Safety and Health, PUI = patient under investigation.

(CDC) are continuously updating the case definitions as new information becomes available (14). The World Health Organization (WHO) suspected, and case definitions can be accessed at Global Surveillance for human infection with COVID-19 (15). Screening protocols should be adapted to the local situation (16, 17).

SPECIMEN REQUIREMENTS FOR MOLECULAR DIAGNOSTICS

In addition to the upper (oral swab, nasopharyngeal swab [NPS], or/and throat swab) (Fig. 2) and lower (sputum, bronchoalveolar

lavage, pleural fluid, lung tissue) respiratory tract (18–20), the presence of the virus has been detected in blood, stool, and anal swabs in the later stages of infection (21–23), suggesting viral shedding and transmission may occur through the oral-fecal route (24, 25). The duration and frequency of shedding of COVID-19 virus RNA in stool and urine is still being established. Only 15% of patients hospitalized with pneumonia had detectable SARS-CoV-2 RNA in serum (5) (although cell-free or unencapsulated RNA is much less stable than DNA in serum). SARS-CoV-2 can also directly infect secondary lymphoid organs (spleen and lymph nodes), and viral nucleocapsid protein can be found in macrophages (26). Blood is currently being primarily used for serologic testing, as well as autopsy samples and will be discussed below. Within 5–6 days of the onset of symptoms, SARS-CoV-2 RNA has been detected in about 32% of oropharyngeal swabs, compared with 63% detection from NPS (see section on false-negatives below). Therefore, the U.S. CDC and other regulatory agencies around the globe recommend collecting the upper respiratory NPS (27, 28). This specimen collection is also better tolerated by the patient and somewhat safer for the healthcare worker to procure. NPS aspirates are also suitable specimens for detection of SARS-CoV-2 when supplies of suitable swabs are limited.

When early NPS may miss the infection, corroborative testing from sputum (29) or oral fluid (saliva) (30) may be appropriate (11). Timing of sampling seems to be critical.

Reports suggest that upper respiratory tract samples may provide higher yield early in the course (0–5 d), whereas lower respiratory tract sampling may have higher yield later in the course of disease (27, 31). In the future, corroborative testing may be required for confirmation of SARS-CoV-2 infections (32–34). Alternatively, successive testing may be considered because the likelihood of the SARS-CoV-2 being present in the nasopharynx increases. After collection, NPS should be placed in viral transport media for immediate delivery (ideally refrigerated) to the testing laboratory site. Stability and ability to detect virus with alternative medias other than viral transport

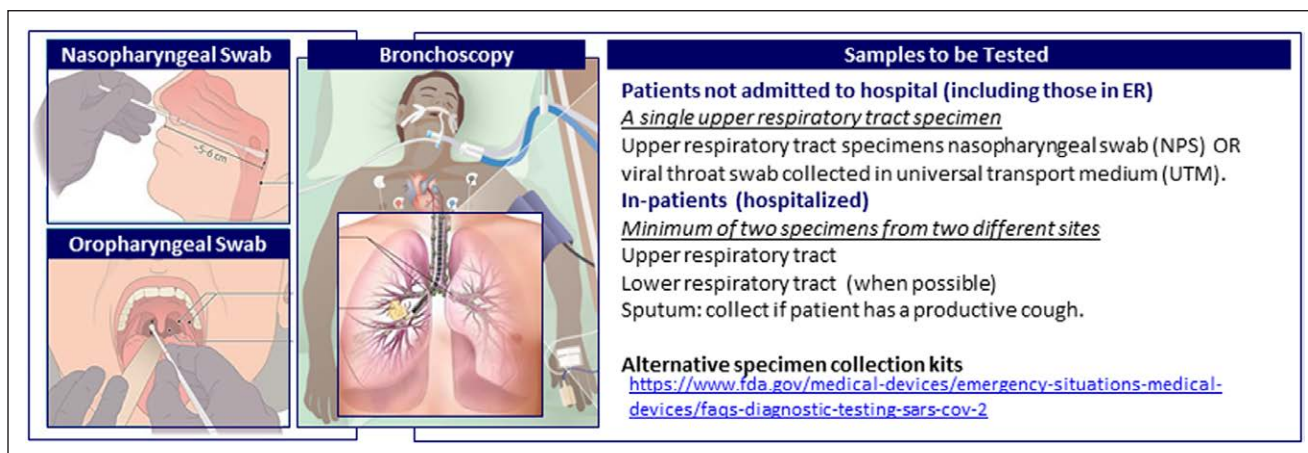


Figure 2. Specimen requirements for molecular diagnostics. Samples from the upper respiratory tract: Nasal- and oral-pharyngeal swabs and/or lower respiratory (bronchoalveolar lavage, endotracheal tube aspirates, pleural fluid, or/and lung tissue samples) should be used for molecular diagnostics. Types and numbers of samples required for screening versus inpatient management are different (<https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-criteria.html>). Swab specimens should be placed in universal or viral transport medium (UTM). ER = emergency room.

medias (saline, protein expression medium, universal transport media) has been demonstrated and approved (35).

BASIC UNDERSTANDING OF VIRAL STRUCTURE AND LIFE CYCLE

The newly identified SARS-CoV-2 genome shares between 74.5% and 85% nucleic acid sequence identity with the SARS-CoV (responsible for the severe acute respiratory syndrome epidemics) (5, 14). Similarities among Coronaviridae family members, including middle east respiratory syndrome coronavirus (36), led initial characterization of the virus genome and molecular diagnostic assay designs. SARS-CoV-2 is an enveloped positive sense single-stranded RNA virus. The genome (37) (26–32 kb) contains at least 10 open reading frames (ORFs) (38) (**Fig. 3A**). The first two ORFs encode nonstructural proteins (nsp1–nsp16) which form the viral replicase transcriptase complex (39). The other ORFs encode the following four structural proteins: spike (S), envelope (E), nucleocapsid (N), and membrane (M), as well as several accessory proteins with unknown functions (**Fig. 3B**) (40). The S protein is responsible for binding and entry into host cells (41). The host receptor for viral entry is the angiotensin converting enzyme 2 (42), expressed in the surface epithelium of the lungs and other organs (43). The virus enters the cells by endocytosis, viral RNA is released into the cytoplasm, translated, and replicated. Newly formed envelope glycoproteins and nucleocapsid are assembled, viral particles germinate into the endoplasmic reticulum-Golgi intermediate compartment. Vesicles containing virus particles then fuse with the plasma membrane to release the virus (44). New viral particles are then shed. Detection of the pathogen involves reliable molecular or serologic detection of viral particles or/and components.

The full genome sequence of SARS-CoV-2 (45), responsible for COVID-19, was released online January 10, 2020 (https://www.ncbi.nlm.nih.gov/nucleotide/NC_004718), just weeks after the disease was first identified in Wuhan, China (46). As the virus spreads around the world, over 4,798 genome sequences have been deposited on Global Initiative on Sharing All Influenza Data (GISAID) (<https://www.gisaid.org/>). A week after the first sequence was

published, the laboratory of Christian Drosten, along with academic collaborators in Europe and Hong Kong, published the sequence of the real-time reverse transcription polymerase chain reaction (RT-PCR) diagnostic test and workflow which detects SARS-CoV-2 and distinguishes it from SARS-CoV (47). The specificity of the primers was verified in isolates or patient samples from 297 patients with various other respiratory infections. This formed the basis of shipments of 250,000 kits, which the WHO dispatched to 159 laboratories across the globe. This PCR-based protocol has since been selected by the WHO as the standard for molecular diagnosis (21), but it is not being widely used in the United States. To illustrate key principles, we focused on the U.S. Food and Drug Administration (FDA) approved protocol (**Fig. 3**) (48, 49)

NUCLEIC ACID AMPLIFICATION TESTS FOR COVID-19 VIRUS

Detection of COVID-19 has received a “strong recommendation” from the recently published rapid advice guideline for the diagnosis and treatment of SARS-CoV-2–infected pneumonia (14). SARS-CoV-2 RNA detection can be achieved using various approaches including real-time RT-PCR, digital droplet polymerase chain reaction (PCR), next generation sequencing, clusters of regularly interspaced short palindromic repeats (CRISPR)-based Specific High Sensitivity Enzymatic Reporter UnLOCKing (50), and employing metagenomic nucleic acid analysis as a routine diagnostic and surveillance tool. PCR, however, is the gold standard for diagnosing an infectious agent and simply implemented—even though “real world” sensitivity and specificity remain to be determined. The novel SARS-CoV-2 genome has a unique sequence about 1,378 nucleotide base pairs long that is not found in other related coronaviruses (38). Three protocols are predominantly in current use (51): the U.S. Centers for Disease Control and Prevention (CDC); the Charité algorithm (Berlin, Germany) (49); and the University of Hong Kong Li Ka Shing Faculty of Medicine protocol (52). Randomized trials evaluating and comparing their performance in the field are required.

TABLE 1. Centers for Disease Control and Prevention 2019–Novel Coronavirus Approved Real-Time Reverse Transcription Polymerase Chain Reaction Diagnostic Primer Panel

Names	Description	Oligonucleotide Sequence (5'→3')
2019-nCoV_N1-F	2019-nCoV_N1 forward primer	5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1-R	2019-nCoV_N1 reverse primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1-P	2019-nCoV_N1 probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'
2019-nCoV_N2-F	2019-nCoV_N2 forward primer	5'-TTA CAA ACA TTG GCC GCA AA-3'
2019-nCoV_N2-R	2019-nCoV_N2 reverse primer	5'-GCG CGA CAT TCC GAA GAA-3'
2019-nCoV_N2-P	2019-nCoV_N2 probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'
2019-nCoV_N3-F	2019-nCoV_N3 forward primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'
2019-nCoV_N3-R	2019-nCoV_N3 reverse primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'
2019-nCoV_N3-P	2019-nCoV_N3 probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'
RP-F	RNAse P forward primer	5'-AGA TTT GGA CCT GCG AGC G-3'
RP-R	RNAse P reverse primer	5'-GAG CGG CTG TCT CCA CAA GT-3'
RP-P	RNAse P	5'-FAM-TTC TGA CCT GAA GGC TCT GCG CG-BHQ-1-3'

nCoV = novel coronavirus.

Available at: <https://www.cdc.gov/coronavirus/2019-ncov/laboratory/rt-pcr-panel-primer-probes.html>.

During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe oligonucleotide, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal (Fig. 3, *D* and *E*). With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence detection allows detection of viral sequences in clinical samples. Multiplex RT-PCR, the simultaneous detection of multiple targets in a single reaction well, using different pairs of primers and probe oligonucleotides for each target is now the desired approach (<https://ourworldindata.org/covid-testing>). Further quantitative and qualitative tests are described at <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations>.

Approved strategies rely on RT-PCR designed to detect unique viral sequences in respiratory specimens during the acute phase of infection (54, 55). Two, one-step quick RT-PCR tests targeted both the open reading frame 1b (ORF1b) and the N regions of the viral genome (Fig. 3A). Initial tests were explicitly designed to identify multiple viruses in the sarbecovirus subgenus to which SARS-CoV-2 belongs, given a lack of data on the genetic diversity of SARS-CoV-2 in humans and animals (56). The N gene assay is recommended as a screening test, and the ORF1b test is recommended as a confirmatory test. Tests typically take 4–6 hours to complete, but the logistical requirement to ship clinical samples means the turnaround time is 24 hours at best. Currently, there are various commercial tests available for the diagnosis of COVID-19; multiple countries have independently developed their own approach. Commercial molecular assay developers such as Roche Diagnostics, Thermo Fisher Scientific, Qiagen (recently acquired and soon to be merged with Thermo Fisher) have received emergency use authorizations (EUA) from the U.S.

FDA. Large reference laboratories in the United S such as Quest Diagnostics and LabCorp are ramping up the capacity to perform large throughput versions of these tests including automated SARS-CoV-2 testing systems and services. The FDA has published approved RT-PCR diagnostic panels that can be used for the diagnosis of COVID-19 (57, 58).

Several U.S. hospitals have developed their own laboratory-developed tests and received FDA EUA for their use in evaluating patient specimens and reporting results to support clinical care decisions. Notably, many research and clinical laboratories were designing tests to detect and diagnose infections with SARS-CoV-2, but multiple hurdles in the U.S. regulatory oversight system had to be overcome to allow these laboratories to proceed. Initially, the FDA would only allow testing using the CDC designed test by CDC or other public health laboratories (PHLs). Delays were compounded by problems with test verification (prior to clinical specimen testing), which included problems with primers, indeterminate results, and kit redesign and replacements (21, 59). This situation has now been rectified with the many EUAs subsequently issued with kits manufactured by many companies making their way into local hospital clinical laboratories. Some sites find that they are able to provide quicker results than the national reference laboratories which now are overwhelmed with a large number of samples and backlogs of more than a week to return results to physicians.

Important Considerations

A comprehensive list of tests for the diagnosis of COVID-19 is available from Foundation for Innovative New Diagnostics (FIND) (this is constantly being updated <https://www.finddx.org/covid-19/pipeline/>). Note that most of the tests on the FIND list are designated CE-IVD which is not suitable for implementation in U.S.

clinical laboratories under U.S. Federal regulations of FDA and Centers for Medicare & Medicaid Services. **Supplemental Table 1** (Supplemental Digital Content 1, <http://links.lww.com/CCX/A282>) lists all commercially available kits as well as kits under development as of March 24, 2020. Most strategies rely on amplifying specific and unique parts of the viral genome (Fig. 3) (19). Pan-coronavirus assays use degenerate primers (60), some use multiple primer sets (61), and others employ a single set of nondegenerate primers (62). Current molecular respiratory panels that detect the endemic human coronaviruses (HCoV) (HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E) require multiple sets of PCR oligonucleotides. SARS-CoV-2 cases tested negative for endemic HCoVs included in molecular respiratory panels (63–65). The Food and Drug Administration (FDA) and Centers for Disease Control (CDC) have made available lists of approved primers that can be used for the diagnosis of COVID-19 (Fig. 3) (66). Preferred targets include ORF1a/b (RNA-dependent RNA polymerase), E, and N (47). The WHO has posted several RT-PCR protocols for detection of SARS-CoV-2 RNA (15).

Positive results are indicative of the presence of SARS-CoV-2 RNA (67); clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status (68). Importantly, RT-PCR alone is a presumptive test result; WHO recommends confirmation with Sanger sequencing, but for many laboratories, this is too time and resource consuming. Positive results do not rule out bacterial infection or coinfection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. These should be considered in the context of the origin of the sample and the known prevalence of virus in the area sampled (i.e., oropharyngeal vs nasopharyngeal), as well as the timing in the evolution of the disease. Negative results should be considered in light of the clinical observations, patient history, and epidemiologic information (11). As with other tests, false-positive results may occur. Repeat testing or testing with different device technologies may be indicated, especially if results are indeterminate or discordant with other aspects of the clinical presentation. For the most part, initial testing is qualitative and does not provide a quantitative value of the number of virions present in the original sample although RT-PCR provides a cycle threshold value which is surrogate for viral load (69).

POINT-OF-CARE DETECTION OF SARS-COV-2 RNA

Although the pre-PCR specimen preparation may require hands-on manipulation, the real-time PCR is usually a single tube, homogeneous assay that requires no post-PCR manipulation to analyze results. The fluorescent signal is captured as the PCR amplification proceeds and detected by the PCR instrument in real-time. On March 21, the FDA issued the first EUA for a point-of-care (POC) COVID-19 diagnostic for the Cepheid Xpert Xpress SARS-CoV-2 test (19, 70). Specialized equipment was developed to permit faster POC devices that can perform the work load in minutes. This real-time PCR assay uses a single disposable cartridge containing all required reagents, which automates nucleic acid preparation from a clinical sample, performs real-time PCR detection, and reports

multiple test results in about 32 minutes—setting the standard for POC testing (71, 72).

FALSE-NEGATIVES AND THE EVOLVING VIRAL GENOME

Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of COVID-19 have been reviewed (73). Briefly, a false-negative result may occur if a specimen is collected, transported, or handled inappropriately. False-negative results may also occur if amplification inhibitors are present in the specimen; frequently, these inhibitors are introduced when inappropriate sample collection devices are employed. For example, dacron-tipped NPS are recommended because cotton tipped swabs usually have inhibitory residue from their production. More complex reasons that may require further troubleshooting include false-negatives that may occur if the virus is present at a level below the analytical sensitivity of the assay or if the virus has acquired genomic mutations, insertions, deletions, or rearrangements that diverge from the PCR primer sequences or if the test is performed too early in the course of illness when levels of viremia are very low. The limit of detection (LoD) for most of the assays used in viral detection is determined to be the lowest detectable concentration of quantitated extracted viral genomic RNA (copies/mL) at which greater than or equal to 95% of all replicates test positive. For the most part, the LoD for reasonably acceptable assays is 500 copies/mL (54).

VARIATIONS IN THE SARS-COV-2 VIRAL SEQUENCES

Over the length of its ~30,000-base-pair genome, SARS-CoV-2 accumulates an average of about one to two mutations per month—the estimated mean evolutionary rate for 2019-nCoV ranges from 1.7926×10^{-3} to 1.8266×10^{-3} substitutions per site per year (74). Variations in viral sequences may affect genomic data surveillance and impact public health in terms of guiding prevention efforts to reduce COVID-19 transmission in real-time (75). Importantly, since no mutations have been observed in over 120 available SARS-CoV-2 sequences (as of February 21, 2020) derived from B cell and T cell epitopes from patients with SARS-CoV-2; preferential diagnostic and immune targeting of these sequences may potentially offer protection against the appearance of novel virus sequences. Accordingly, key assays have focused on amplifying ORF1 and/or 2 and the S or N protein assuming that these sequences will be essential and conserved, so that the PCR primers and probes will still be effective. Correlation between viral genomes and clinical phenotype among different “strains” based on existing genomic analysis is fraught with methodological errors, including founder effect (76). Furthermore, recent reports suggest genomic sequences of SARS-CoV-2 assembled from patients and GISAID demonstrated stable evolution, similar virulence leading to similar clinical outcomes. Importantly, differences in disease severity are likely linked to host factors such as age, lymphocytopenia, and associated cytokine storm (77).

DETECTION OF COVID-19 USING CRISPR DIAGNOSTICS

Trace amounts of specific RNA can be detected using the gene-editing technique CRISPR (50). These tests rely on the CRISPR machinery: a bacterial enzyme that has a piece of “guide” RNA riding with it that attaches only to specific stretches RNA. The ability to label specific RNA strands allows for rapid detection (78). To check for the presence of COVID-19 RNA in nucleic acid extractions of specimens, various companies have chosen different targets in the COVID-19 genome including the *S* gene and *Orf1ab* gene. Recombinase polymerase amplification primers and Cas13a from *Leptotrichia wadei* (LwaCas13a) CRISPR guide RNAs were designed for specific detection. In order to maximize the specificity of the assay, guide sequences that minimize off-targets to related human respiratory virus genomes have been developed. These tests are being fast-tracked for diagnostic use (79).

ANTIGEN-BASED RAPID DIAGNOSTIC TESTS

Rapid antigen diagnostic tests are suitable for POC testing since they directly detect the presence or absence of an antigen. This distinguishes it from other tests that detect antibodies (antibody tests) or nucleic acid (nucleic acid tests). In the past, these tests have suffered from poor sensitivity—as experienced with this method for detection of influenza viruses (80–82). It is also unclear when in the course of disease are these tests most useful, and in the United States, the EUA has not been granted. A preprint online from Diao et al (83) claimed detection of nucleocapsid protein of SARS-CoV-2 in NPS and urine samples within 10 minutes. Results of nucleocapsid protein positive and negative participants were 100% concordant with nucleic acid test from the same samples. However, these findings are currently unpublished and their utility untried at the bedside.

IMMUNOASSAYS AND SEROLOGIC TESTS

Immunoassays exploit antibody-antigen recognition, either by using monoclonal antibodies (mAbs) to detect viral antigens in clinical samples or by using cloned viral antigens to detect patient antibodies directed against the virus. Serology is not appropriate for initial diagnosis of COVID-19 because the antibodies may not be detected until 14 days post infection. These tests may provide historic information about past viral exposure, as well as diagnostic evidence of infection. In general, immunoassays are both less sensitive and less specific than PCR-based tests and take longer to develop, but they are easy to use and deliver results in 20–60 minutes. Since immunoassays detect patient antibodies to a pathogen, these inevitably must contend with the inherent variability of the human polyclonal antibody response, as well as the delay required to mount humoral immunity. Yang An-Suei Laboratory research fellow at the Genomics Research Center, Academia Sinica, in Taipei, Taiwan, is developing a monoclonal antibody (mAb) against the N protein of SARS-CoV-2, which could form the basis of a rapid antigen test. Such tests detect both immunoglobulin (Ig) M and IgG antibodies, which are released during the initial and later stages of an infection, respectively. During the early stage of the infection (days 4–10), the IgM component of

the test provides a sensitivity of just 70% (24). This rises rapidly to 92.3% between days 11 and 24, and the IgG component of the test offers a sensitivity of 98.6% during this phase of the infection. Overall, the test has a false-negative rate of 13% (84). Halifax, Nova Scotia-based Sona Nanotech, is using the S1 domain of the SARS-CoV-2 spike protein as the basis of an antigen-based lateral flow immunoassay (LFIA) in cooperation with GE Healthcare Life Sciences (85). Cellex has received U.S. FDA EUA for a LFIA to detect IgM and IgG in blood serum, plasma, or venipuncture whole blood. It is like a pregnancy test and results can be read in 15 minutes with 94% pregnancy protein assay, 96% nasopharyngeal aspirate (57). Tests to detect polyclonal antibodies against SARS-CoV-2 in patients are quicker to develop than tests to detect the virus itself. Berlin-based Pharmacy has already started shipping a 20-minute immunoassay containing three SARS-CoV-2 antigens: the N protein and the S1 and S2 domains of the S protein. The tests only incorporate immobilized recombinant viral antigen (which is much easier to generate than mAbs against the virus) and offer similar levels of accuracy to mAb-based tests (86). The Immunoassays and Serologic Test space is currently expanding quickly, and updated information can be accessed from the IDSA's Serology primer website (87).

VIRAL ISOLATES AND CULTURE

Isolation and culture of SARS-CoV-2 is not routinely performed for diagnostic purposes. The main limitation includes the absence of permissive cell catheters, time to results, labor and expertise requirements, significant biosafety risks for laboratory personnel, and the lack of commercial antisera for culture confirmation (19, 88).

PROTOCOLS AND GUIDELINES FOR TESTING

Biosafety practices in the laboratory testing on clinical specimens from patients meeting the suspected case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances. There is still limited information on the risk posed by COVID-19, but all procedures should be undertaken based on a risk assessment. Specimen handling for molecular testing would require biosafety level (BSL)-2 or equivalent facilities. Attempts to culture the virus require BSL-3 facilities. The WHO has published specific guidelines for handling COVID-19 specimens (86, 89)

SUMMARY AND CONSIDERATIONS

As the world is struggling to contain the COVID-19 outbreak, healthcare infrastructure and testing capacity have emerged as major issues. On February 11–12, 2020, WHO organized a forum (<https://www.who.int/news-room/detail/12-02-2020-world-experts-and-funders-set-priorities-for-covid-19-research>) to identify research gaps and priorities for COVID-19, in collaboration with the Global Research Collaboration for Infectious Disease Preparedness (<https://www.glopid-r.org/>) (90). Recognition of the urgent need for access to accurate and standardized diagnostics for SARS-CoV-2 which can be deployed in decentralized settings

was identified as a key priority. A Research and Development Roadmap for COVID-19 was published in March 2020 (90).

Adequate testing capacity for SARS-CoV-2 is lacking worldwide, preventing people from accessing care. An interactive map (91) to track the number of SARS-CoV-2 tests that have been performed in each country is being developed by FIND. This novel tool allows governments and public health officials to compare the number of positive results, at a global and country level. The WHO is continually updating technical guidance for COVID-19 (15, 16), including recommendations on laboratory testing. Our understanding of SARS-CoV-2 prevalence in most populations is primarily limited to detection in patients presenting with symptoms of COVID-19. Testing of mildly affected individuals who have not presented for medical care and of asymptomatic individuals who may be carrying and spreading SARS-CoV-2 has generally not been made available in most countries. These aspects must be characterized through public health testing and contact tracing to understand overall patterns of transmission and risk for future SARS-CoV-2 outbreaks.

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The authors have disclosed that they do not have any potential conflicts of interest.

Address requests for reprints to: C. C. dos Santos, MSc, MD, Clinician-Scientist, Associate Professor of Medicine, Interdepartmental Division of Critical Care, St. Michael's Hospital/University of Toronto, 30 Bond Street, Room 4-008, Toronto, ON, M5B 1WB, Canada. E-mail: dossantosC@smh.ca

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