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Laboratory diagnostics methods of Covid 19 (Coronavirus) an update from the global perspective

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Abstract
Severe acute respiratory syndrome coronavirus (SARS-CoV)-2, a novel coronavirus from the same family as SARS-CoV and Middle East respiratory syndrome coronavirus, has spread worldwide leading the World Health Organization to declare a pandemic[1]. The disease caused by SARS-CoV-2, coronavirus disease 2019 (COVID-19), presents flu-like symptoms which can become serious in high-risk individuals[2]. Here, we provide an overview of the known diagnostics method for COVID-19. We carried out a systematic literature search using the main online databases (PubMed, Google Scholar, MEDLINE, Up-to-date, Embase and Web of Science) with the following keywords: 'COVID-19', '2019-nCoV', 'coronavirus' and 'SARS-CoV-2' Biomarkers'. We included publications from 1 January 2019 to 3 April 2021 which focused on laboratory diagnosis. We found that Real-time PCR is used as a diagnostic tool using nasal swab, throat swab, tracheal aspirate or Broncho alveolar lavage samples. Computed tomography findings are important for both diagnosis and follow-up.
Key Words: *Covid 19, SARS, RT PCR, Corona virus.*

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I. Introduction:

Coronavirus (CoV) is a large family of positive-sense, single-stranded RNA viruses that belong to the Nidovirales order. The order includes Roniviridae, Arteriviridae, and Coronaviridae families. The Coronaviridae family is subdivided into Torovirinae and Coronavirinae subfamilies. Coronavirinae is further subclassified into alpha, beta, gamma, and delta CoVs. Phylogenetic clustering accounts for the classification of these subtypes of viruses[1].

The disease is called corona virus disease 2019 (COVID-19) by the World Health Organization (WHO). The virus causing it is a coronavirus (COV or CoV) of the beta-CoV lineage. The current outbreak of a novel coronavirus is causing many deaths among vulnerable members of our societies and health care professionals. The viral infections can lead to a severe acute respiratory syndrome (SARS). The virus behind the outbreak was named 2019-nCoV in an early research report and SARS-CoV-2 by the International Committee on Taxonomy of Viruses[2].

Epidemiology:

On 31 Dec. 2019, China, East Asia, most populated country in world was informed to WHO regarding pneumonia cases with unknown etiology. On 7 Jan2020, Chinese research authorities were announced that they were isolated new virus from sea food market in Wuhan city, Named as 2019-nCoV the ministry of health, labor and welfare Japan were reported first case imported from Wuhan China. On 20 Jan. 2020, National IHR Focal point from the Korea was reported first case 2019-nCoV in Korea. On 23 Jan. 2020, United State of America were confirmed first case of 2019-nCoV in America. On 24 Jan. 2020, Vietnam has reported First case of 2019-nCoV with not travel history from China,. On 24 Jan. 2020, the government of Singapore was confirmed First case of 2019-nCoV. On 25 Jan. 2020, the government of Australia, federal democratic republic of Nepal and French republic were confirmed first of 2019-nCoV. Other countries also were detected and reported the cases of 2019-nCoV as On, 26 Jan. 2020 (Malaysia), 27 Jan. 2020 (Canada), 28 Jan. 2020 (Cambodia, Germany, Sri Lanka), 29 Jan. 2020 (United Arab Emirates), 30 Jan. 2020 (Philippines, India , Finland), 31 Jan. 2020 (Italy), 1 Feb. 2020 (Russian Federation, Spain, Sweden, United Kingdom), 5 Feb. 2020 (Belgium), 6 Feb. 2020 (Japan), 15 Feb. 2020 (Egypt)[3].

Etiology:

Human coronaviruses (HCoVs) spread in a similar fashion as Rhinoviruses, by direct contact with infected secretions or large aerosol droplets . Health care workers are at increased risk of acquiring COVID-19 infection, possibly due to direct contact with the patients. Indeed, transmission of HCoVs through environmental contamination has been reported in healthcare settings . Understanding which are the potentially contaminated surfaces in a healthcare environment is crucial to protect healthcare workers from this virus showing an unprecedented exponential trend with a doubling time of 3.6-4.1 days . In this regard, studies suggest that surfaces and suspensions can carry HCoVs, increasing the risk of contact transmission that couldlead to hospital acquired HCoVs infections. Otter et al. found that other coronaviruses (SARSCoV, MERS-CoV) can be found on plastic, metal and cloths for up to 6 days. Thus, monitoringenvironmental contamination of SARS-CoV-2 can support investigation of the current outbreak andbenefit the management of COVID-19 infection. environmental contamination with SARS-CoV-2through respiratory droplets and fecal shedding suggests that the environment is indeed a potential
medium of transmission.[4]

II. Methods:

Using online databases, we carried out a systematic literature review of the clinical features of and treatments for the new COVID-19. Key articles were retrieved mainly from PubMed, Google Scholar, MEDLINE, UpTo Date, Embase and Web of Science, using the terms 'COVID-19', '2019-nCoV', 'coronavirus' and 'SARS-CoV-2' as keywords for our search. We included scientific publications from 1 January 2019 to 3 April 2020. Only publications focusing on clinical characteristics of and treatments for SARS-CoV-2 were eligible for inclusion. We screened all reference lists of relevant studies in order to identify any missing publications.

All searches as well as title and abstract screening and study selection were performed by two investigators working independently. We resolved any discrepancies through consensus. All articles deemed potentially eligible were retrieved for full-text review. We limited our search results to publications in English and excluded abstracts from conferences and commentaries.

Laboratory Features:

Laboratory findings specific to COVID-19 include elevated prothrombin time, LDH (lactate dehydrogenase), D-dimer, ALT, C-reactive protein (CRP), and creatine kinase . In the early stages of the disease, a marked reduction in CD4 and CD8 lymphocytes can also be noted. Patients in the intensive care unit have shown higher levels of interleukin (IL) 2, IL-7, IL-10, GCSF (granulocyte colony-stimulating factor), IP10 (interferon gamma-induced protein 10), MCP1 (monocyte chemotactic protein 1), MIP1A (macrophage inflammatory protein alpha), and TNF-α (tumor necrosis factor-α) . They also displayed other abnormal findings indicative of coagulation activation, cellular immune deficiency, myocardial injury, renal injury, and hepatic injury . In critical patients, amylase and D-dimer levels are significantly elevated . However, blood lymphocyte counts progressively decreased . Common to non-survivors are the elevations in ferritin, neutrophil count, D-dimer, blood urea, and creatinine levels . Elevations in procalcitonin levels are not a feature of COVID-19. Therefore, an elevated level of procalcitonin may suggest an alternative diagnosis such as bacterial pneumonia. Levels of CRP correlate directly with disease severity and progression[5].

Gene analysis:

The single-stranded RNA genome of the 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) about 29.9 kb in length and encoding about 9860 amino acids, was annotated to possess 14 open reading frames (ORFs) and 27 proteins1,2. The orf1ab and orf1a genes at the 5′-terminus of the genome encode the pp1ab and pp1a proteins, respectively, together form 15 non-structural proteins (nsps), nsp1-nsp10 and nsp12-nsp16. The 3′-terminus of the genome encodes four structural proteins, the spike surface glycoprotein (S), the small envelope protein (E), membrane protein (M) and nucleocapsid protein (N). There are eight accessory proteins denoted as 3a, 3b, p6, 7a, 7b, 8b, 9b and ORF142[6].

Sample collection:

Nasopharyngeal (NP) swabs can be used for testing asymptomatic persons in a healthcare setting, including long-term care facilities. At this time anterior nares and mid-turbinate specimen collection are only appropriate for symptomatic patients and both nares should be swabbed. The guidance below addresses options for collection of specimens once a clinical determination has been made to pursue SARS-CoV-2 testing.

For initial diagnostic testing for SARS-CoV-2, CDC recommends collecting and testing an upper respiratory specimen. Nasopharyngeal specimen is the preferred choice for swab-based SARS-CoV-2 testing. When collection of a nasopharyngeal swab is not possible, the following are acceptable alternatives:

- An oropharyngeal (OP) specimen collected by a healthcare professional, or
- A nasal mid-turbinate (NMT) swab collected by a healthcare professional or by onsite self-collection (using a flocked tapered swab), or
- An anterior nares (nasal swab; NS) specimen collected by a healthcare professional or by onsite self-collection (using a flocked or spun polyester swab)

- Nasopharyngeal wash/aspirate or nasal aspirate (NA) specimen collected by a healthcare professional

For NS, a single polyester swab with a plastic shaft should be used to sample both nares. NS or NMT swabs should be placed in a transport tube containing either viral transport medium, Amies transport medium, or sterile saline. See the standard operating procedure for public health labs to create viral transport mediumpdf icon in accordance with CDC's protocol. both NP and OP swabs are collected, they should be combined in a single tube to maximize test sensitivity and limit use of testing resources.

CDC also recommends testing lower respiratory tract specimens, if available. For patients who develop a productive cough, sputum should be collected and tested for SARS-CoV-2. The induction of sputum is not recommended. When it is clinically indicated (e.g., those receiving invasive mechanical ventilation), a lower respiratory tract aspirate or bronchoalveolar lavage sample should be collected and tested as a lower respiratory tract specimen.

Specimens should be collected as soon as possible once a decision has been made to pursue SARS-CoV-2 testing, regardless of the time of symptom onset. Maintain proper infection control and use recommended personal protective equipment, which includes an N95 or higher-level respirator (or facemask if a respirator is not available), eye protection, gloves, and a gown, when collecting specimens.

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious disease. A specimen that is not collected correctly may lead to false negative test results. The following specimen collection guidelines follow standard recommended procedures.

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 mL of viral transport media. In general CDC is now recommending collecting only the NP swab. If both swabs are used, NP and OP specimens should be combined at collection into a single vial. OP swabs remain an acceptable specimen type.

Nasopharyngeal swab: Insert flexible wire shaft minitip swab through the nares parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.

Oropharyngeal swab (e.g., throat swab): Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.

Nasopharyngeal wash/aspirate or nasal aspirate (NA)

Attach catheter to suction apparatus. Have the patient sit with head tilted slightly backward. Instill 1 mL-1.5 mL of non-bacteriostatic saline (pH 7.0) into one nostril. Insert the tubing into the nostril parallel to the palate (not upwards). Catheter should reach depth equal to distance from nostrils to outer opening of ear. Begin gentle suction/aspiration and remove catheter while rotating it gently. Place specimen in a sterile viral transport media tube.

Nasal mid-turbinate (NMT) swab, also called Deep Nasal Swab

Use a flocked tapered swab. Tilt patient's head back 70 degrees. While gently rotating the swab, insert swab less than one inch (about 2 cm) into nostril (until resistance is met at turbinates). Rotate the swab several times against nasal wall and repeat in other nostril using the same swab. For more information, see the CDC Influenza Specimen Collectionpdf icon instructions. Note that these instructions are applicable for respiratory viruses in general, and not specific for influenza virus.

Anterior nares specimen (NS)

Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nares and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nares with same swab.

Bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy
Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/or fatal cases.

Sputum

Educate the patient about the difference between sputum and oral secretions (saliva). Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below. If specimens will ship without delay, store specimens at 2-8°C, and ship overnight to CDC on ice pack. If a delay in shipping will result in receipt at CDC more than 72 hours after collection, store specimens at -70°C or below and ship overnight to CDC on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

Label each specimen container with the patient's ID number (e.g., medical record number), unique CDC or state-generated nCov specimen ID (e.g., laboratory requisition number), specimen type (e.g., serum) and the date the sample was collected. Complete a CDC Form 50.34 for each specimen submitted. In the upper left box of the form, 1) for *test requested* select "Respiratory virus molecular detection (non-influenza) CDC-10401" and 2) for *At CDC, bring to the attention of* enter "Stephen Lindstrom: 2019-nCoV PUI."[9]

Diagnostic methods:

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.25 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-CoV2 have been identified, but the host response to the virus is still under investigation. The first genome sequence of SARS-CoV2 was conducted with metagenomic RNA sequencing, an unbiased and high-throughput method of sequencing multiple genomes.26−28 The findings were publicly disclosed, and the sequence was added to the GenBank sequence repository on January 10, 2020.26,27 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.29,30 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 2020.2,4 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.31 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 diagnosing COVID-19.32 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.33,34 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.35 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 highthroughput 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.36 This assay format is more sensitive than the one-step assay, but it is more timeconsuming and requires optimizing additional parameters.36,37 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-2.38 However, RT-PCR is the most predominantly used method for diagnosing COVID-19 using respiratory samples.2,39 Upper respiratory samples are broadly recommended, although lower respiratory samples are recommended for patients exhibiting productive cough.40 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.41,42 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.3,43 Winichakoon et al. recommended multiple lines of evidence for patients linked epidemiologically even if the results are negative from nasopharyngeal and/or oropharyngeal swab.43The United States Centers for Disease Control and Prevention (CDC) uses a one-step real time RT-PCR (rRTPCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-CoV-2.44 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).32 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.44 During rRTPCR, 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.44 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 SARSCoV-2.45 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.45 See Table 1 (Charité, Germany). Chu et al. proposed a slightly different assay workflow.46 They screened samples using primers for the N gene and used those from the ORFlb gene for confirmation. A diagnosis where the patient sample is positive with N gene primer and negative with the ORFlb gene would be inconclusive. In such situations, protein tests (i.e., antibody tests) or sequencing would be required to confirm the diagnosis.46 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 COVID19.47 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.48,49 The images are analyzed by radiologists to look for abnormal features that can lead to a diagnosis.48 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)50 with a maximum lung involvement peaking at around 10 days after the onset of symptoms.51 The most common hallmark features of COVID-19 include bilateral and peripheral ground-glass opacities (areas of hazy opacity)52 and consolidations of the lungs (fluid or solid material in compressible lung tissue).50,51 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 followed by increasing consolidation of the lungs.50,51 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.3,25,53,54 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.3 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. 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.56 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-2.57−60 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.61 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).62 The reaction occurs in <1 h at 60− 65 °C with an analytical limit of detection of ∼75 copies per μL. The approach is simple to operate, easy to visualize for detection, has less background signal, and does not need a thermocycler.61 The drawbacks to LAMP are the challenges of optimizing primers and reaction conditions. Other isothermal amplification techniques for COVID-19 detection are in development.62 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.63 Multiplexing increases the amount of information gained from a single test and improves clinical sensitivity and specificity.64 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.65,66 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. 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.68 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.69 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.70 A SHERLOCK protocol for detecting SARSCoV-2 has been released,71 and another Cas13a-based detection system has been tested with SARS-CoV-2 clinical isolates.72 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.73 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.74 Currently, serological tests (i.e., blood tests for specific antibodies) are in development.75−77 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).75 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. 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. 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%.76 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.78 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).79 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.80 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.81 These technologies can be adapted to detect SARS-CoV-2 RNA or proteins.

Smartphone Surveillance of Infectious Diseases. Controlling epidemics requires extensive surveillance, sharing of epidemiological data, and patient monitoring.82,83 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).84,85 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-19.84. 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.100 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.101 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-19.102 Mudanyali et al. also developed a smartphone-based microscope that transfers diagnostic results to a database for analysis and spatiotemporal mapping.103 These devices can help address the need for point-of-care testing at the community level, where there is underreporting[10].

Laboratory findings:

General guidelines for interpreting COVID-19 real-time RTPCR* *Intended for commercial kits with EUA as of March 25, 2020 Criteria for determining positive and negative results in the screening and confirmatory tests Positive control (+), negative control (-) - Threshold cycle (Ct) value of the target gene ≤cut-off Ct value: positive for the gene*. - No target gene detected or Ct value >cut-off Ct value: negative for the

gene*. *Independent of internal control amplification All results of negative control (+) or positive control (-) Invalid regardless of target gene and internal control amplification; retest is necessary. See details below. Note: Values close to the cut-off values in specimens with low viral loads may indicate false-negative or false-positive results. Thus, a laboratory physician should interpret the results and if necessary, retest using residual or new specimens. Criteria for final test interpretation All kits currently available in Korea can detect two or more genes. According to the interpretation criteria of some manufacturers, detection of only one of multiple genes is interpreted as COVID-19 positive. However, based on results from actual clinical specimens, KSLM recommends a determination of a positive result only when all genes are detected. When only one gene is detected, retesting or consulting the reference laboratory is recommended. 1) Screening test (+) and confirmatory test (+): positive for COVID-19 (SARS-CoV-2 detected). Among the reagents with EUA, some kits with three target genes use one target gene for the screening test and the other two target genes for the confirmatory test. For these kits, the confirmatory test result is deemed positive only if both confirmatory genes are detected. If one gene is not detected, the result cannot be interpreted as positive. 2) Screening test (+) and confirmatory test (-): negative for COVID-19 (SARS-CoV-2 not detected). For kits using betacoronavirus primers for the screening test, there is a possibility of betacoronavirus rather than SARS-CoV-2. 3) Screening test (-) and confirmatory test (-): negative for COVID-19 (SARS-CoV-2 not detected). If the internal control is also negative, the result is invalid, and a retest is necessary. 4) Screening test (-) and confirmatory test (+): retest or refer to a reference laboratory for additional testing. Considerations At present, there is very limited knowledge regarding the timing of virus detection in COVID-19 cases. Hence, it is difficult to rule out COVID-19 based solely on one negative result, especially when using an upper respiratory tract specimen from a suspected case. Other upper respiratory tract specimens should be collected and tested when a highly suspected COVID-19 patient tests negative using a single upper respiratory tract specimen. The lower respiratory tract specimens may be collected together and tested. Some considerations for possible false negative results are listed below:

Possible causes of false negative results [4, 26] - Inadequate specimen quality. - Specimens collected too early or too late. - Specimens improperly handled or transported. - Occurrence of viral genetic mutation. - Presence of PCR inhibitors. - Antiviral administration prior to testing. Solutions - If the upper respiratory tract specimens test negative, lower respiratory tract specimens should be collected and tested. - Patient specimens, a positive control, and a negative control should be examined together, and internal controls should be examined and verified together in all reactions. - If a patient with an epidemiological correlation and COVID-19 symptoms repeatedly tests negative, the tested specimen should be submitted to the KCDC for further testing[11].

The laboratory abnormalities predominantly found included hypoalbuminemia, elevated inflammatory markers, such as C-reactive protein, LDH, and ESR, among others. Also, lymphopenia is consistently present in more than 40% of the patients across eight studies with more than 500 patients. Data from the 2002–2003 outbreak indicate that SARS may be associated with lymphopenia, leukopenia, and thrombocytopenia, elevated levels of LDH, alanine transaminase (ALT), AST, and creatine kinase [54,55], but also, and not significantly seen, nor consistently reported, in COVID-19 studies and cases, with thrombocytopenia, mild hyponatremia, and hypokalemia. The frequency of lymphopenia found suggests that COVID-19 might act on lymphocytes, especially T lymphocytes, as does SARS-CoV, maybe including depletion of CD4 and CD8 cells [4]. Virus particles spread through the respiratory mucosa, initially using the ACE2 receptor at ciliated bronchial epithelial cells, and then infect other cells. This induces a cytokine storm in the body and generates a series of immune responses, that cause changes in peripheral white blood cells and immune cells such as lymphocytes [12].
Common laboratory findings include normal/ low white cell counts with elevated C-reactive protein (CRP)[13].

III. Discussion:

During the early phase of the coronavirus disease 2019 (COVID-19) pandemic, design, development, validation, verification and implementation of diagnostic tests were actively addressed by a large number of diagnostic test manufacturers. Hundreds of molecular tests and immunoassays were rapidly developed, albeit many still await clinical validation and formal approval. In this Review, we summarize the crucial role of diagnostic tests during the first global wave of COVID-19. We explore the technical and implementation problems encountered during this early phase in the pandemic, and try to define future directions for the progressive and better use of (syndromic) diagnostics during a possible resurgence of COVID-19 in future global waves or regional outbreaks. Continuous global improvement in diagnostic test preparedness is essential for more rapid detection of patients, possibly at the point of care, and for optimized prevention and treatment, in both industrialized countries and low-resource settings)[14].Rapid and early laboratory diagnosis of COVID-19 is the main focus of treatment and control. Molecular tests are the basis for confirmation of COVID-19, but serological tests for SARS-CoV-2 are widely available and play an increasingly important role in understanding the epidemiology of the virus and in identifying populations at higher risk for infection. Point-of-care tests have

the advantage of rapid, accurate, portable, low cost and non-specific device requirements, which provide great help for disease diagnosis and detection[15].

Clinically, the diagnosis of this unprecedented illness, called coronavirus disease-2019 (COVID-19), becomes difficult because it shares many symptoms with other respiratory pathogens, including influenza and parainfluenza viruses. Therefore, laboratory diagnosis is crucial for the clinical management of patients and the implementation of disease control strategies to contain SARS-CoV-2 at clinical and population level[16]. In the preanalytical stage, collecting the proper respiratory tract specimen at the right time from the right anatomic site is essential for a prompt and accurate molecular diagnosis of COVID-19. Appropriate measures are required to keep laboratory staff safe while producing reliable test results. In the analytic stage, real-time reverse transcription-PCR (RT-PCR) assays remain the molecular test of choice for the etiologic diagnosis of SARS-CoV-2 infection while antibody-based techniques are being introduced as supplemental tools. In the postanalytical stage, testing results should be carefully interpreted using both molecular and serological findings. Finally, random-access, integrated devices available at the point of care with scalable capacities will facilitate the rapid and accurate diagnosis and monitoring of SARS-CoV-2 infections and greatly assist in the control of this outbreak[17].Human-to-human transmission via droplets, contaminated hands or surfaces has been described, with incubation times of 2-14 days. Early diagnosis, quarantine, and supportive treatments are essential to cure patients. Treatments, including antiviral agents, chloroquine and hydroxychloroquine, corticosteroids, antibodies, convalescent plasma transfusion and vaccines.Current diagnostic tests for coronavirus include reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR (rRT-PCR), and reverse transcription loop-mediated isothermal amplification (RT-LAMP). RT-LAMP has similar sensitivity to rRT-PCR, is highly specific and is used to detect MERS-CoV. According to current diagnostic criteria founded by the China National Health Commission, laboratory examinations, including nasopharyngeal and oropharyngeal swab tests, have become a standard assessment for diagnosis of COVID-19 infection. To identify patients earlier, two one-step quantitative RT-PCR (qRT-PCR) assays were developed to detect two different regions (ORF1b and N) of the SARS-CoV-2 genome. Three novel RT-PCR assays targeting the RNA-dependent RNA polymerase (RdRp)/helicase (Hel), spike (S), and nucleocapsid (N) genes of SARS-CoV-2 were developed. Among the three novel assays, the COVID-19-RdRp/Hel assay had the lowest limit of detection in vitro; highly sensitive and specific assays may help to improve the laboratory diagnosis of COVID-19[18].Reliable laboratory diagnosis represents one of the main tools for the promotion, prevention, and control of infectious diseases1 . The diagnostic methods for COVID-19 fall under two main categories: immunological and molecular. Immunological tests can be serological tests that mainly detect antibodies in blood or viral antigens in respiratory secretions, and both can be performed with point-of-care platforms. Regarding molecular tests, they are based on the detection of SARS-CoV-2 RNA mainly in nasopharyngeal samples, which in most cases require adequate laboratory infrastructure. In addition to the cited tests, other laboratory parameters have been used as an aid in the clinical monitoring.Serological tests are especially important for the diagnosis of patients with mild to moderate disease, in the absence of molecular diagnostics. These tests can have several benefits, such as estimating the transmissibility and lethality rates, assessing individual and community immunity, and valuing the need and effectiveness of nonpharmaceutical interventions (e.g., social isolation). Furthermore, the plasma of convalescents with high levels of antibody production could be used as a therapeutic support. Several serological tests based on enzyme-linked immunosorbent assay (ELISA), and lateral flow immunochromatography (LFI) devices have been developed by different companies worldwide. IgM and IgG antibodies detected on ELISA have more than 95% specificity in the diagnosis of COVID-19 . High titers of IgG antibodies detected by ELISA demonstrate a positve correlation with neutralizing antibodies[19]. Many in-house and commercial diagnostic kits have been developed or are under development that have a potential to lower the burden of diagnosis on the primary diagnostic techniques like RT-PCR. Serological based diagnosis is another broad category of testing that can detect different serum antibodies like IgG, IgM, and IgA in an infected patient.

The Truenat Beta CoV E-gene screening assay and Truenat SARS-CoV-2 RdRp gene-confirmatory assay (Molbio Diagnostics, India) were earlier validated as a two-step test.The assays were deployed for COVID-19 testing in various parts of India between April and June, 2020.A multiplex assay combining E-gene screening and Orf1a-gene confirmatory assay has also been validated recently. All three of these assays exhibited 100% sensitivity and specificity, and positive and negative predictive value when compared with the gold-standard RT-PCR test[20].PCR-based diagnostic procedures that are commonly used for pathogen detection need sophisticated machines and assistance of a technical expert. Despite their reliable accuracy, they are not cost-effective tests, which a common man can afford, so it becomes imperative to look for other diagnostic approaches, which could be cost effective, rapid, and sensitive with consistent accuracy. To make such diagnostics available to the common man, many techniques can be exploited among, which are Point of Care (POC), also known as bed side testing, which is developing as a portable and promising tool in pathogen diagnosis. Other lateral flow assay (LFA)-based techniques like SHERLOCK, CRISPR-Cas12a (AIOD-

CRISPR), and FNCAS9 editor limited uniform detection assay (FELUDA), etc. have shown promising results in rapid detection of pathogens. Diagnosis holds a critical importance in the pandemic situation when there is no potential drug for the pathogen available in the market[20].

Although research is still in its early stages, the discovery of how different biomarkers behave during the course of the disease could help clinicians in identifying severe disease earlier and subsequently improve prognosis. Nevertheless, we urge for more research across the globe to corroborate these findings.

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