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The Diagnosis of Covid-19 Infection Pandemic: Current Issues and Challenges

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Abstract: *SARS-CoV-2 or COVID-19 is an enveloped RNA virus that is diversely originated among humans and in wildlife. Mainly, six types of species have been recognized to cause disease in humans. They are acknowledged to affect there respiratory, hepatic, enteric, and neurological systems in humans. The SARS-CoV-2 or COVID-19 outbreak had a key effect on clinical microbiology research laboratories in the past quite a few months. However, CT imaging along with electron microscopy were primarily used to identify and screen COVID-19 and also the biological etiology of SARS-CoV-2. This review covers recent issues along with challenges for the laboratory diagnosis of infections caused by SARS-CoV-2. This will introduce a general outline of COVID-19 and describe the symptoms, modes of transmission along with biological properties of COVID-19 or SARS-CoV-2. It will also provide a means to increase consciousness among primary and secondary healthcare workers during the recent crucial pandemic situation. Moreover, our analysis focuses on the most up-to-date clinical information for the effective diagnosis tools of COVID-19 patients globally.*

Key Words: *COVID-19, CT, RNA virus, Respiratory, Neurological system, Genome sequencing, Laboratory diagnosis.*

1. INTRODUCTION:

The novel coronavirus disease 2019 (COVID-19) presents a very important and urgent threat to global health.A cluster of patients with unknown cause of pneumonia was identified in Wuhan , China, in early December 2019[1]. A cluster of patients with fever, cough , shortness of breath and other symptoms is admitted[2]. Subsequently, the causative pathogen was classified as a severe acute respiratory syndrome-related coronavirus-2 (SARS – CoV-2)[3], a newly defined betacoronavirus. This virus, now known as the etiologic agent of COVID-19 disease, is the seventh known coronavirus to infect humans[1]. About 36,000 peoples are dead from infection with COVID-19 (up to 30 March 2020)[3]. The world Health Organization registered more than 9, 26, 000 cases in more than 195 countries, regions, or territories as of 1 April 2020[4].Samples from patients' bronchoalveolar lavage (BAL) fluid were analyzed by 10 January 2020. This showed the lineage of betacoronavirus B to a pathogen with the same genetic code. It was discovered that this pathogen had ~80 percent ,~50 percent , and ~96 percent similarity with the genome of the severe acute respiratory syndrome virus (SARS-CoV), Middle East respiratory syndrome virus (MERS-CoV), and bat coronavirus RaTG13, respectively [5,6]. On 11 February 2020, the World Health Organization proposed that the novel viral infectin be called "Corona Virus Disease (COVID19)," while the International Committee on Virus Taxonomy (ICTV) proposed the name as "SARS-CoV-2" due to the phylogenetic and taxonomic study of this novel coronavirus[8]. While the number of people infected with COVID-19 continues to grow globally and healthcare services are becoming increasingly stressed, it is clear that the clinical laboratory will play an significant role in this crisis, contributing to patient screening , diagnosis, monitoring / treatment, and epidemiological recovery / surveillance. This chapter focuses on the genetic structure, infection source, transmission route,clinical characteristics, and diagnostic testing as well as emerging diagnostic outcome of the SARS-CoV-2 (COVID-19), so that it can provide references for follow-up research, prevention, and treatment, and can help readers to have the advanced understanding of this novel infectious disease.Within this review article, we object to summarizing the existing recognized biological properties of SARS-CoV-2, diagnostic methods and clinical results for the identification of SARS-CoV-2, FDA policy to control the spread. This is an significant research subject, and a review article that discusses the latest findings can be beneficial in developing strategies to tackle the emerging COVID-19 pandemic around the world.

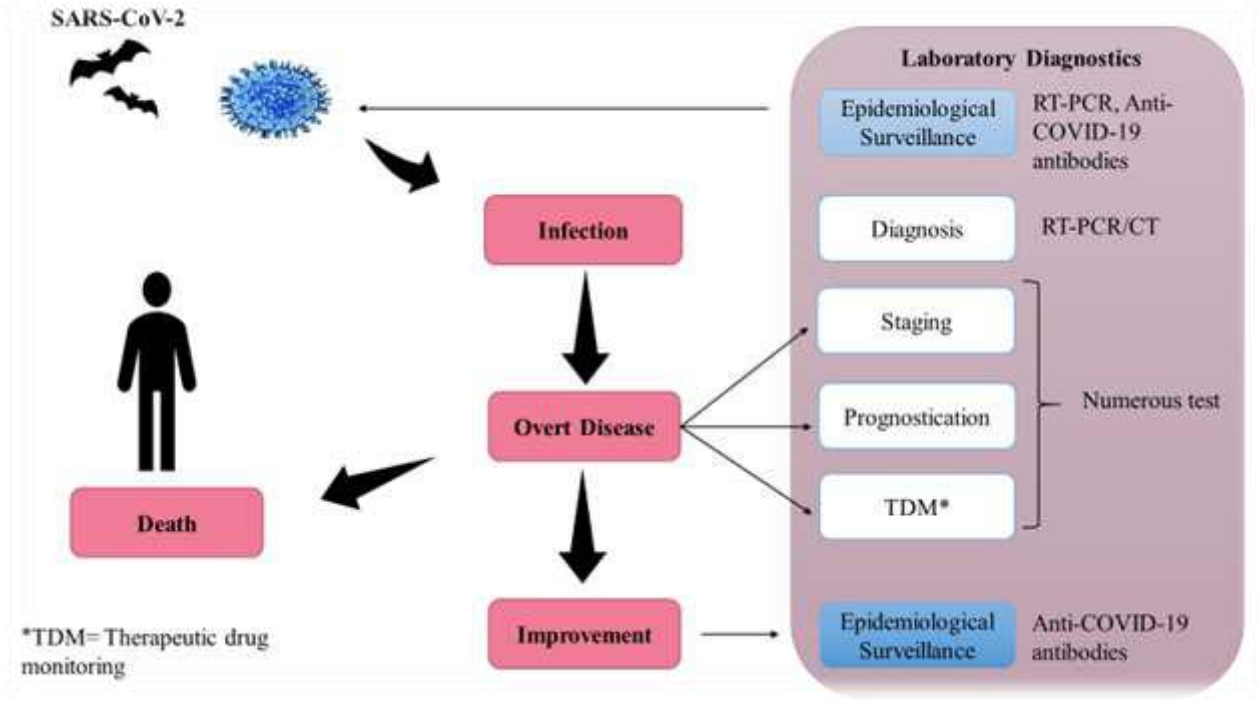

Figure 1. The crucial role of laboratory diagnostics in SARS-CoV-2 (COVID-19) infection

2. SYMPTOMS:

The COVID-19 (SARS-CoV-2) virus affects totally different people in dissimilar ways. Most infected people can develop gentle to moderate symptoms.According to rumours, cases of confirmed coronavirus disease 2019 (COVID-19) ranged from mild symptoms to severe sickness and death.On average it takes 5–6 days from once somebody is infected with the virus for symptoms to point out, but it will take up to two weeks in some cases.Few common symptoms specific to SARS-CoV-2 include low-grade fever that gradually increases with shortness of breath, with cough that gets severe illness over time. Doctors as well as scientist, both of are still learning new things concerning this virus daily. So far, we all know that SARS-CoV-2 might not initially cause any symptoms for a few people. However, some people might expertise with new symptoms as well as runny nose, nasal congestion, diarrhea, aches, and pains. Older people, and people with underlying medical issues like high blood pressure, heart issues or diabetes, have more probability to develop serious sickness. Most people (about 80%) get over the disease while not having special treatment.Approximately one out of every six people who get SARS-CoV-2 (COVID-19) get severely unwell and experience breathing difficulties.

3. MODES OF TRANSMISSION:

Droplets of various sizes can spread respiratory or lung infections: if the droplet particles are greater than 5-10 μm in diameter and less than 5 μm in diameter, they are referred to as droplet nuclei[8].According to present evidence, the SARS-CoV-2 virus is mainly transmitted between humans through respiratory droplets as well as contact routes [2,9-13].In an study of 75,465 COVID-19 (SARS-CoV-2) cases in China, the aerial diffusion was not shown to be the cause. Droplet transmission occurs when a person is in close contact (within 1 m) with someone with respiratory problems (e.g., coughing or sneezing) and is therefore at risk of exposure by his / her mucosa (mouth and nose) or conjunctive (eyes) to infectious respiratory droplets. Transmission around the infected individual may also occur via fomites inside the immediate environment[14]. The transmission of the COVID-19 virus may therefore occur due to direct contact with an infected individual and indirect contact with surfaces within the immediate environment or with devices used by the infected person (e.g., stethoscope or thermometer). Airborne transmission varies from droplet transmission as it refers to the presence of microorganisms inside droplet nuclei that are regarded as particles In the case of COVID-19, the transmission mechanism is also possible under various conditions and settings in which aerosol-generating processes or support treatments are performed, i.e. Endotracheal intubation, bronchoscopy, nebulized treatment administration, pre-intubation manual ventilation, placing the patient into a prone position, disconnecting the subject from the ventilator, non-invasive positive-pressure ventilation, tracheostomy, and cardiopulmonary resuscitation. Some indications are there that COVID-19 infection may cause intestinal infection and also be observed in faeces. One report exists in the literature about the culturing of the COVID-19 virus from one stool specimen [15]. There are no reports of faecal−oral transmission of the COVID-19 virus to date.Studies have pointed out that 2019-nCoV is also airborne through aerosols formed during medical procedures[16].Notably, 2019-nCoV RNA could also be

detected through rRT-PCR testing in a stool specimen collected on day 7 of the patient's disease[17]. However, the aerosol transmission route and the fecal-oral transmission route concerned still need a lot of studies.

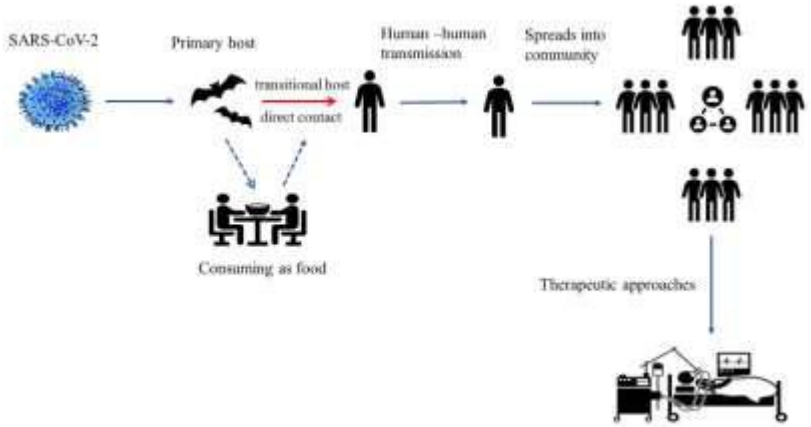

Figure 2. The mode of transmission of SARS-CoV-2 (COVID-19) infection

4. BIOLOGICAL PROPERTIES OF SARS-CoV-2 (COVID-19):

All coronaviruses belong to the family of *Coronaviridae*, of the order *Nidovirales*, comprising large, single, plus-stranded RNA as their genome [18,19]. Presently, there are divided into four genera of coronaviruses: α-CoV, β-CoV, γ-CoV, and δ-CoV[20,21]. Most of the coronavirus can cause infectious diseases in humansalong with vertebrates also. The α-CoV and β-CoVmostly infect the respiratory, central nervous system, and gastrointestinal tract of humans along with mammals, while γ-CoV and δ-CoVmostly infect the birds [18,22,23,24]. Usually, numerous members of the coronavirus cause mild respiratory symptoms in humans; however, SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) explored in 2002–2003 and in 2012, separately, caused fatal severe respiratory diseases [25,26,27].The SARS-CoV and MERS-CoV belong to the β-CoV group[28,29]. 2019-nCoV (COVID-19 or SARS-CoV-19)discovered in Wuhan, China also belongs to the β-CoV according to the phylogenetic analysis based on the viral genome [5,30]. Although the similarity among nucleotides is less than 80% between 2019-nCoV and SARS-CoV (about 79%) or MERS-CoV (about 50%), 2019-nCoV also can cause infection in the foetus and spread faster than the two other coronaviruses. [5,11,31,32,33,34]. The identical genome nucleotide between a coronavirus (BatCoV RaTG13) detected within the bat *Rhinolophus affinis* from Yunnan, China, and 2019-nCoV was 96.2%, which indicates that the natural host of 2019-nCoV can also be the *Rhinolophus affinis* bat[5]. However, the differences also suggest that there can be more intermediate hosts between the bat and human. A research team from the South China Agricultural University has investigated 1000 metagenomic samples from pangolins and found that 70% pangolins contained β-CoV [35].One of the pangolin-isolated coronaviruses consisted of a genome that was found to be very close to that of 2019-nCoV, and the similarity of the genome sequence was 99 percent, suggesting that the pangolin could be the intermediate host of 2019-nCoV[36]. The supernatant was obtained from damaged or killed cells and examined by electron microscopy with negative-stained transmission[37]. The photographs showed that the virus has a diameter from 60 to 140 nm and is enclosed in an envelope with protein spikes and genetic material[1]. The general structure looks just about like other Coronaviridae family viruses.cellstaken from humans were cultured with the virus from BAL fluid isolated from patients. The structure of SARS-CoV-2 includes a single-stranded positive-sense RNA genome that's ~30,000 nucleotides long [5,38]. The genome encodes 27 proteins including an RNA-dependent RNA polymerase (RdRP) and four structural proteins. [38,39]. RdRP acts in combination with nonstructural proteins to retain genome fidelity. A region of the RdRP gene in SARS-CoV-2 was almost like a RdRP gene found in the RaTG13 bat coronavirus and 96 per cent like the overall genome sequence of RaTG13[5]. Of the 104 strains sequenced between December 2019 and mid-February 2020, a similar sequence was found at 99.9 percent, but more recently improvements within the viral genome have been cataloged, indicating a greater variety of sequences[2,41]. SARS-CoV-2 's four structural proteins include spike surface glycoprotein (S), a small protein envelope (E), matrix protein (M), and nucleocapsid protein (N). The S gene codes for the receptor-binding spike protein in coronaviruses which enable the virus to infect cells[42]. This spike protein facilitates binding of the receptor and fusion of the membrane which determines the capabilities of host tropism and transmission[6]. The S gene is divergent in SARS-CoV-2 compared to all previously mentioned SARS-related coronaviruses with a similarity of less than 75 per cent nucleotide sequence[5]. The remaining three structural proteins are more conserved than the spike protein and are required for general coronavirus function[38]; these proteins are involved in RNA enclosure and/or protein assembly, budding, envelope formation, and pathogenesis[44-46] . SARS-

CoV-2 interact with the angiotensin-converting enzyme 2 (ACE2) receptor for entry into cells. Zhou *et al*. conducted studies by incubating SARS-CoV-2 with HeLa cells of differential ACE2 receptor expression [5].The author of fluorescently stained viruses has shown co-localization with cells that express the ACE2 receptor from Chinese horseshoe bats, pigs, humans and civets but not from mice.ACE2 mRNA 's presence is amongst all human organs. ACE2 exists in the lungs, stomach , small intestine, colon, skin, lymph nodes, liver bile ducts, kidney parietal epithelial cells, and brain, in arterial and venous endothelial cells and arterial smooth muscle cells. It is also expressed on the surface of small intestine lung alveolar epithelial cells and enterocytes that allow them to become infected. Tissues of the upper respiratory tract (i.e., oral and nasal mucosa and nasopharynx) did not exhibit ACE2 surface expression on epithelial cells and were the primary site of SARS-CoV-2 infection[47].By understanding the biological properties of SARS-CoV-2 researchers can develop diagnostics can be built for detection.

5. DIAGNOSTIC TESTING FOR 2019-nCoV:

In the absence of effective therapeutic drugs or vaccines for COVID-19, early identification of the disease and prompt isolation of an infected patient from a healthy population is important. The symptoms expressed by the patients with COVID-19 are unspecific. Can not be used for a accurate diagnosis. Guan et al . reported that 44 percent of China's 1099 COVID-19 patients had a fever when they entered the hospital, and that 89 percent had a fever while in hospital[46]. Two approaches were used for diagnostic testing of the novel coronavirus 2019-nCoV: Whole genome sequencing and Real-time reverse transcriptase PCR (rRT-PCR). Sequencing was used mainly for the primary detection of this novel virus in the early days of the outbreak, and is mainly a viral discovery tool. Nearly every diagnostic test for nCoV is currently done using rRT-PCR. The diagnosis of COVID-19 must be confirmed, by reverse transcription polymerase chain reaction (RT-PCR) or gene sequencing for respiratory or blood specimens, as a key indicator for hospitilization. according to the latest guidelines published by the Chinese Government. However, with sample selection and transportation limitations as well as kit results, the overall positive RT-PCR rate for throat swab samples was stated at the initial presentation to be about 30 per cent to 60 per cent[47,48].

5.1. GENOME SEQUENCING:

Now that the virus has been identified, to characterize the virus and monitor for viral mutation, not only clinical diagnosis, most sequencing is undertaken to further research. But some sequencing is also being done to generate epidemiological information in real-time. Next-generation sequencing (NGS) can be a valuable tool for characterizing and detecting viruses in the environment, in animals, and humans during viral pandemics[49]. Next-Generation Sequencing (NGS) test is an NGS-based test to detect SARS-CoV-2 through several types of samples such as Nasal Swab, Nasopharyngeal Swab, Oropharyngeal Swab, Extracted RNA.The NGS method, also called deep sequencing, uses more comprehensive laboratory and computational methods compared to standard methods (RT-PCR), to detect the virus that causes COVID-19. This test also characterizes the whole viral genome, in addition to detecting the virus. SARS-CoV-2 sequence analysis plays a significant role in understanding the viral evolution, the properties of viruses, and therapeutic drug production. The data that this test produces can be used to help researchers learn more about the existence of this virus. Usually tests are available within 2-4 days of obtaining the sample from the lab.

5.2. PCR (RT-PCR):

The key method for diagnosing SARS-CoV-2[50] is the testing of nucleic acid. A few numbers of the reverse transcription-polymerase chain reaction kits (RT-PCR) were designed to genetically detect SARS-CoV-2. RT-PCR involves reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by extension of specific cDNA regions[51,52]. In general, the design process includes two main steps: (1) sequence alignment and primer design, and (2) optimization and testing of assays. Corman et al. aligned and analyzed a variety of sequences of the viral genome associated with SARS in order to construct a series of primers and probes. [54]. Among the SARS-related viral genomes, scientists have discovered three regions that had conserved sequences: (1) the RdRP gene (RNA-dependent polymerase gene) in the ORF1ab region, (2) the E gene (protein gene envelope), and (3) the N gene (protein gene nucleocapside). All the RdRP and E genes have a high analytical sensitivity for detection (technical detection limit of 3.6 and 3.9 copies per reaction), while the N gene is less sensitive to analytics (8.3 copies per reaction). The evaluation can be designed as a two-target system, where one primer detects numerous coronaviruses universally including SARS-CoV-2 and a second primer set detects SARS-CoV-2 only.The United States Centers for Disease Control and Prevention (CDC) is using a one-step real-time RT-PCR (rRT-PCR) assay to detect the presence of SARS-CoV-2[55], which offers quantitative information on viral loads. The viral RNA is extracted to conduct the assay and added to a master mix. The master mix includes nuclease-free water, forward and reverse primers, a fluorophore-quencher probe, and a reaction mix (which consists of reverse transcriptase, polymerase, magnesium, nucleotides, and additives)[50]. The master mix and extracted RNA are loaded into a PCR thermocycler, and the temperature of the incubation is set to run the assay. The CDC had proposed rRT-PCR cycling conditions[55]. Corman et al. have projected a three-step workflow for the SARS-CoV-2 diagnosis [56]. The three steps are defined as screening, confirmation, and discriminatory assays. To maximize the number of patients identified as infected, the first step detects all SARS-related viruses by targeting

various regions of the E gene. If this test is positive, then they suggest RdRP gene detection using two different primers and two different probes. If those results are also positive, they carry out the discriminatory test with one of the two sequences of the probes. [57]. Chu et al. suggested a slightly different method for testing [57].They screened samples using N gene primers and used those for confirmation from the ORFlb gene. A diagnosis where the group of patients is positive with the primary gene N and negative with the gene ORFlb will be inconclusive. In such situations, the diagnosis [57] would require protein tests (i.e., antibody tests) or sequencing.

5.3. COMPUTED TOMOGRAPHY (CT):

Due to the lack of kits and the false-negative rate of RT-PCR in the Hubei Province, China used CT scans as a clinical diagnosis for COVID-19[57] for a temporary period. Chest CT scans are non-invasive and involve taking multiple X-ray measurements across a patient's chest from different angles to produce cross-sectional images [58,59]. Radiologists examine the images to look for anomalous characteristics that could lead to diagnosis [58]. COVID-19 's imaging features are diverse and depend on the stage of infection following symptom onset. For example, in the early stages of the disease (0–2 days) Bernheim et al. saw more frequent normal CT findings (56 percent)[60] with a maximum lung involvement peaking at around 10 days after the onset of symptoms[61]. Chest CT, a routine imaging device for pneumonia diagnosis, is fast and comparatively easy to perform. Recent research found that the sensitivity of CT for COVID-19 infection was 98% compared to RT-PCR sensitivity of 71% [76]. Some literature on radiology suggests a pivotal role in CT. Ai and his colleagues[62] report on 1014 patients who received both RT-PCR and CT during their epidemic in Wuhan, China. Researchers found that 97% of patients with RT-PCR-confirmed diagnoses had CT pneumonia findings and concluded that "CT imaging is extremely prone to COVID-19 diagnosis." Some reports are less optimistic. Inui and colleagues[63] studied CT scans from the Diamond Princess Cruise liner on 112 RT-PCR-confirmed COVID-19 cases. Less than two thirds (61 percent) of cases had lung opacities on CT; negative CTs were observed in 20 percent of symptomatic patients.

6. EMERGING TESTS FOR COVID-19:

It is very important to emphasize that different tests serve different determinations in the management of this pandemic situation: while point-of-care testing, protein testing along with ancillary diagnostic tests, acute detection of those infected with SARS-CoV-2 will be increasingly valued as time goes by on the potential of immunological testing for contact tracing, with efforts to produce them

6.1. POINT-OF-CARE IMMUNODIAGNOSTIC:

Point-of-care (POCT) testing is essential for the rapid detection of analytes close to the patient, which facilitates better diagnosis, monitoring, and management of diseases. It allows for quick medical decisions, as the diseases can be diagnosed at a very early stage, resulting in improved health outcomes for patients by allowing early treatment to begin. One point-of-care method under progress for the treatment of SARS-CoV-2 is the lateral flow antigen detection[64]. A paper-like membrane strip is coated with two lines in commercial lateral flow assays: gold nanoparticle-antibody conjugates are present in one line, and antibodies are captured in the otherThe sample of the patient (e.g., blood and urine) is deposited on the membrane, and capillary action draws the proteins across the strip. When the first line moves, the antigens bind to the nanoparticle-antibody gold conjugate, and the complex flows across the membrane together. The Capture Antibodies immobilize the complex when they enter the second side, and a red or blue line is visible. Individual gold nanoparticles are red, but the coupling of the plasmon band allows a blue solution that includes clustered gold nanoparticles. The lateral flow test showed 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 yield a clinical sensitivity of 82%[64]. Microfluidic devices are another approach for use at the point of care. These devices consist of a palm-sized chip etched with channels and reaction chambers of the micrometer scale. The chip uses electrokinetic, capillary, vacuum, and/or other forces to mix and separate the liquid samples. These chips can be made from materials like polymethyl sulfoxide, glass, or paper. Miniaturization, low sample volume, fast detection times, and portability are the main advantages of using microfluidics [65]. In order to improve point-of-care monitoring, rapid point-of-care assays for SARS–CoV-2 on instruments would be critical. The Xpert Xpress SARS – CoV-2 test (Cepheid) has accepted an EUA FDA and is performed on the GeneXpert platform, previously commonly used for tuberculosis (TB) and HIV testing, particularly in low- and middle-income countries. This capability could be useful for extending testing worldwide as well as in settings where rapid results at the point of care would allow for clinical decisions, although testing performance may be a limiting factor

6.2. PROTEIN TESTING:

Viral protein antibodies and antigens that are formed in response to infection with SARS-CoV-2 may be used to diagnose COVID-19. Changes in viral load can make it difficult to detect viral proteins throughout the infection. For example, in the first week after symptom onset, Lung et al. showed high salivary viral loads which gradually declined with time[66]. Antibody tests may be particularly useful for SARS-CoV-19 surveillance. One potential challenge with developing accurate serological tests includes potential cross-reactivity of SARS-CoV-2 antibodies against other

coronaviruses. Another study carried out by Lv et al. tested plasma samples from 15 COVID-19 patients against SARS-CoV-2 and SARS-CoV S proteins and found a high level of cross-reactivity [67]. Serological tests (that is, blood tests for particular antibodies) are currently under progress. [68 to 70]. Nevertheless, Hang et al. used an enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G and M (IgG and IgM) from the human serum of COVID-19 patients [68]. They used the nucleocapsid protein SARS-CoV-2 Rp3, which has 90 percent homology of the amino acid sequence to other SARS-related viruses. Antibodies have been found in samples of respiratory, blood, or fecal. Xiang et al. have also detected antibodies to SARS-CoV-2 IgG and IgM in reported cases[69]. In light of recent research, other protein or cellular markers can also be used for identification. Guan et al. demonstrated high levels of C-reactive protein and D-dimer in infected patients, as well as low levels of lymphocytes, leukocytes, and blood platelets[46]. The challenge of using these biomarkers is that they are anomalous in other diseases, too. A multiplex test may enhance the specificity of both an antibody and small molecule markers.

7. ANCILLARY DIAGNOSTIC TESTS:

The ideal use of biomarkers and other nonmicrobiological tests is rapidly evolving.

7.1. BIOMARKERS CONNECTED WITH COVID-19:

The most popular laboratory features identified in COVID-19 patients include reduced albumin (75.8% [95 % CI, 30.5% to 100%]), elevated rates of lactate dehydrogenase (57.0% [CI, 38.0% to 76.0%]), and elevated C-reactive protein (58.3% [CI, 21.8% to 94.7%]), and lymphopenia (43.1% [CI, 18.9% to 67.3%]). Other biomarkers identified include increased rates of erythrocyte sedimentation; elevated levels of aminotransferase aspartate, alanine aminotransferase, and creatinine kinase; leukopenia; leukocytosis; and increased levels of bilirubin and creatinine[72-74].Such findings are not surprising, since these biomarkers represent an inflammatory host response to SARS – CoV-2 (COVID-19) or are early markers of end-organ dysfunction, similar to those seen in sepsis patients[75]. There are currently no biomarkers or combinations of biomarkers (more than one or two) that are sensitive or accurate enough to establish a COVID-19 diagnosis, or to rationally predict its clinical path.

Table 1. Emerging Diagnostics Being Developed for SARS-CoV-2

Platform	Analytical technique	Point-of-Care (Y/N)	Biomarker	Ref.
Smartphone dongle	ELISA	Y	Protein	77
SIMOA	Digital ELISA	Y	Protein	78
Rapid antigen test	Lateral Flow	Y	Protein	79
Bio barcode assay	DNA-assisted immunoassay	Y	Protein	80
Para-magnetic bead	Magnetic biosensor	N	Protein	81
CRISPR	RPA	Y	Nucleic Acid	82
CRISPR	RT-RPA	Y	Nucleic Acid	83
NASBA	Real-time NASBA	N	Nucleic Acid	84
Magnetic bead	Magnetic	N	Nucleic Acid	85
Quantum dot barcode	Barcode	Y	Nucleic Acid	86

8. VALIDATION STUDY OF DIAGNOSTIC TESTS:

- **MOLECULAR DIAGNOSIS:**SARS-CoV-2 (COVID-19) molecular diagnostic tests as unique tests that detect SARS-CoV-2 nucleic acids from human samples. The following validation studies be led for a molecular SARS-CoV-2 (COVID-19) diagnostic as per FDA authority suggests:

8.1. Limit of Detection (LoD):

The FDA recommends that labs document their SARS-CoV-2 Detection Limit (LoD). Food and Drug Administration (FDA) usually has no problems with spiking RNA or inactivated virus into an artificial or actual clinical matrix for LoD determination (e.g. BLA (Bronchoalveolar Lavage Fluid), sputum, etc.). The Agency suggests that laboratories test a dilution sequence of 3 replicates per concentration, so make sure that the final concentration is 20 replicates. In this guidance, authority determines that LoD is positive as the lowest concentration at the 19/20 replicates. If multiple clinical matrices are supposed for clinical testing, the authority recommends that laboratories submit in their EUA requests the results from the most difficult clinical matrix to FDA. as an example, if testing respiratory specimens

(e.g., sputum, BAL, nasopharyngeal (NP) swabs, etc.), laboratories should include only results from sputum in their EUA request.

8.2. Clinical analysis:

In the absence of identified positive samples for testing, authority advises that laboratories ensure their assay output with a series of designed clinical specimens by evaluating a total of 30 contrived reactive and 30 non-reactive specimens. Country-wide reactive specimens are often created by spiking RNA or inactivated viruses into remaining clinical specimens, most of which are often leftover from higher respiratory specimens such as NP swabs or lower respiratory tract specimens such as sputum, etc. We tend to suggest that twenty of the artificial clinical specimens are spiked at a concentration of 1x-2x LoD, with the remainder of the species spanning the assay testing.For this guidance, FDA defines the acceptance criteria for the performance as of 95th agreement at 1x-2x LoD, and 100 pc agreement in the least alternative concentrations and negative samples.

8.3. Inclusivity:

Laboratories should log the results of an in-silico analysis showing the percentage of identity matches against publicly out there SARS-CoV-2 sequences which the projected molecular analysis may detect. The FDA anticipates that 100 pc of reported sequences of SARS-CoV-2 would be detectable with the selected primers and probes.

8.4. Cross-reactivity:

At a minimum, FDA claims that an in-silico examination of the primer assay and the probes is appropriate for initial clinical use compared to specific respiratory flora and alternative pathogens with infectious agents. In silico cross-reactivity, FDA defines for this guidance a greater than 80th similarity between one in all the primers/probes and any sequence present within the targeted being. In addition, the FDA recommends that laboratories follow recognized laboratory procedures for the sample varieties to be tested for any additional cross-reactivity tests.

- **ANTIGEN DETECTION DIAGNOSTICS**

SARS-CoV-2 antigen diagnostic tests as those that detect SARS-CoV-2 antigens directly from clinical specimens. FDA recommends that the following validation studies be conducted for a SARS-CoV-2 antigen test:

- ✓ Limit of Detection/Analytical Sensitivity
- ✓ Cross-reactivity/Analytical Specificity
- ✓ Microbial Interference
- ✓ Clinical Agreement Study

The purpose of the clinical agreement study is to establish the performance characteristics of the test (e.g., Sensitivity / PPA, Specificity / NPA). Confidence in clinical agreement on human specimens should be recognized by the FDA (Food and Drug Administration), preferably samples left from patients with or without SARS-CoV-2 (COVID-19) infections. If SARS-CoV-2 can't obtain positive clinical samples, spiking leftover samples with SARS-CoV-2 materials is unacceptable. The most challenging matrix should be used in your validation studies for devices that claim multiple clinical matrices.

- **SEROLOGICAL DIAGNOSTICS:** SARS-CoV-2 (COVID-19) serological diagnostic tests as novel tests detecting antibodies (e.g., IgM, IgG) from the clinical samples of SARS-CoV-2 (COVID-19). The FDA recommends conducting the following validation trials for a serological review of SARS-CoV-2

- ✓ Cross-reactivity/Analytical Specificity
- ✓ Class Specificity
- ✓ Clinical Agreement Study

The clinical contract or agreement study aims to create the performance characteristics of the test (e.g., Sensitivity / PPA, Specificity / NPA). The FDA suggests that the clinical accuracy of microbiologically confirmed COVID-19 life-threatening infection on human specimens should be recognized globally.

9. FDA ISSUED POLICY

Inside the U.S. On February 29, 2020, the Food and Drug Administration (FDA) in progress and aggressive obligation to deal with coronavirus (COVID-19) outbreak, the agency allocated a new policy to certain laboratories seeking to develop diagnostic tests for coronavirus to achieve a lot of rapid testing capacity in the United States. This guidance is provided by the Food and Drug Administration (FDA) to include a guideline to help promote the accessibility of new diagnostic tests for coronavirus (COVID-19 or SARS-CoV-2) produced by commercial manufacturers and laboratories during the general emergency as well as pandemic situation globally. The Health and Human Services Secretary (HHS) announced that on February 4, 2020, there was a public health emergency that justified the authorization or approval of emergency use of in vitro diagnostics for the detection and/or treatment of the novel coronavirus (COVID-19). Rapid identification of cases of SARS-CoV-2 (COVID-19) in the U.S. requires robust diagnostic test accessibility to monitor the emergence of this rapidly spreading, serious disease. This guidance defines a policy for both commercial manufacturers and laboratories to help accelerate the use of tests they develop to achieve

a lot of speedy and widespread testing capabilities in the United States.The following guidelines should be conducted for a molecular SARS-CoV-2 diagnostic as per Food and Drug Administration (FDA) suggested a newly issued policy:

- Laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) that meet the CLIA regulatory needs to perform high complexity testing exploitation their validated tests beforeEmergency Use Authorization (EUA) submission
- State Authorization of Laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) that meet the CLIA regulatory needs to perform high complexity testing
- Industrial Manufacturer Development & Distribution of tests before Emergency Use Authorization(EUA) submission
- Industrial Manufacturer Development & Distribution and Laboratory Development and use of serology tests without an Emergency Use Authorization(EUA)

10. CONCLUSION:

The COVID-19 (SARS-CoV-2) pandemic has historically highlighted the crucial role of diagnostics within the management of communicable diseases. Consequently, their performance estimates are probably to be optimistic and deceptive. Upcoming studies should address these considerations. Sharing information and expertise for development, validation, and updating of COVID-19 interrelated prediction models are desperately required. Intensive diagnostics deployment most likely contributed to the success of some countries in dominant transmission like India. Imperative public health as well as clinicaldesires currently drive an unprecedented world effort to extend SARS–CoV-2 (COVID-19) testing capability.

10.1. CONFLICT OF INTEREST STATEMENT: The authors declare that there is no conflict of interest.

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