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MATURING LAB SCALE COVID-19 TECHNOLOGIES FOR TRACKING, TESTING, AND TREATING

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ABSTRACT

Coronavirus disease 2019 (COVID-19) was discovered in Hubei Province, China in December 2019. A cluster of patients were admitted with fever, cough, shortness of breath, and other symptoms. Initial diagnosis of pneumonia was found by Computed Tomography (CT). By 10 January 2020 samples from patients' bronchoalveolar lavage (BAL) fluid were analysed to reveal a pathogen with a similar genetic sequence to the betacoronavirus B lineage. It was discovered that this new pathogen had ~80%, ~50%, and ~96% similarity to the genome of the severe acute respiratory syndrome virus (SARS-CoV), Middle East respiratory syndrome virus (MERSCoV), and bat coronavirus RaTG13, respectively. The novel coronavirus was named SARS-CoV-2, the pathogen causing COVID-19. SARS-CoV-2 can be transmitted from human to human. The current hypothesis is that the first transmission occurred between bats and a yet-to-be-determined intermediate host animal. It is estimated that a SARS-CoV-2-infected person will infect approximately three new people (the reproductive number is averaged to be 3.28). The symptoms can vary, with some patients remaining asymptomatic, while others present with fever, cough, fatigue, and a host of other symptoms. At this stage, the most likely mode of transmission is thought to be through direct contact and droplet spread.

KEYWORDS: SARS-CoV-2, Computed Tomography (CT), bronchoalveolar lavage (BAL).

PURPOSE OF THE DOCUMENT

Depending on the intensity of transmission, the number of cases and laboratory testing and surge capacity, it may be necessary to prioritize who gets tested according to health objectives. WHO has outlined critical priority actions for preparedness, readiness, and response actions for COVID-19 and has defined four transmission scenarios:

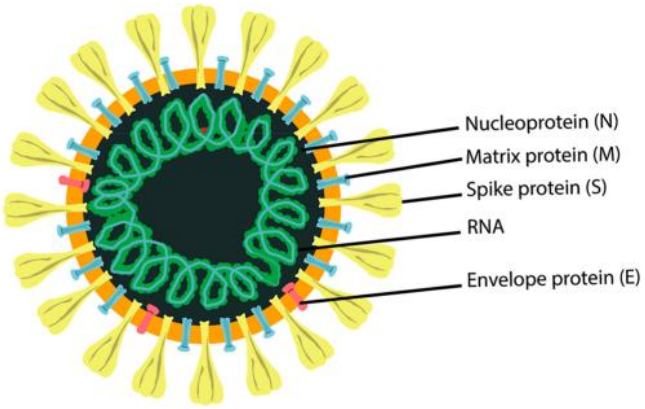
1. Countries with no cases (No Cases);
2. Countries with 1 or more cases, imported or locally detected (Sporadic Cases);
3. Countries experiencing clusters of cases related in time, geographic location, or common exposure (Clusters of cases);
4. Countries experiencing larger outbreaks or sustained and pervasive local transmission (Community transmission).

As part of the Strategic Preparedness and Response Plan, WHO developed testing strategy recommendations. The foundation of this strategy is threefold:-

- All countries should increase their level of preparedness, alert, and response to identify, manage, and care for new cases of COVID-19; laboratory testing is an integral part of this strategy.
- Countries should prepare to respond to different public health recognizing that there is no one-size-fits-all approach to managing cases and outbreaks of COVID-19.
- Each country should assess its risk and rapidly implement the necessary measures at the appropriate scale and prepare for a testing and clinical care surge to reduce both COVID-19 transmission and economic, public health, and social impacts.

Biological Properties of SARS-CoV-2

Human airway epithelial cells were cultured with the virus from BAL fluid isolated from patients. Supernatant was collected from cells that were damaged or killed and analyzed by negative stained transmission electron microscopy. The overall structure looks similar to other viruses from the Coronaviridae family with diameter ranging from 60 to 140 nm, protein spikes envelope and a genetic material.



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

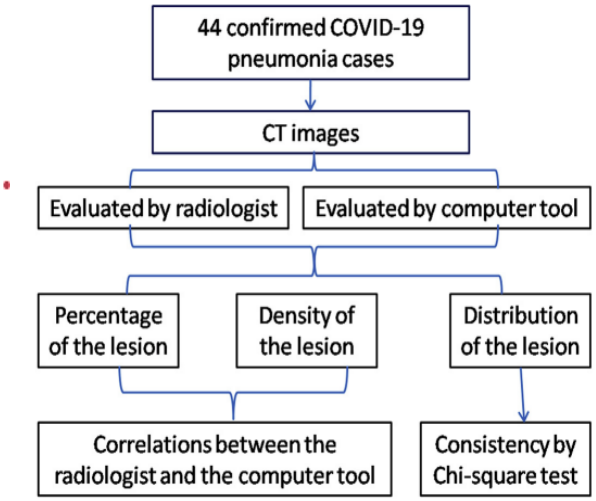
(RdRP) and four structural proteins. The four structural proteins of SARS-CoV-2 include the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N). In coronaviruses, the S gene codes for the receptor-binding spike protein that enables the virus to infect cells. This spike protein mediates receptor binding and membrane fusion, which determines host tropism and transmission capabilities. The proteins other than (S) are involved in encasing the RNA and/or in protein assembly, budding, envelope formation, and pathogenesis. SARS-CoV-2 appears to interact with the angiotensin converting enzyme 2 (ACE2) receptor for entry into cells. The ACE2 mRNA is present in almost all human organs. ACE2 is present in arterial and venous endothelial cells and arterial smooth muscle cells in the lungs, stomach, small intestine, colon, skin, lymph nodes, liver bile ducts, kidney parietal epithelial cells, and the brain. It is also expressed on the surface of lung alveolar epithelial cells and enterocytes of the small intestine that allows them to be infected. Tissues of the upper respiratory tract (i.e., oral and nasal mucosa and nasopharynx) did not show surface expression of ACE2 on epithelial cells and therefore are unlikely the primary site of SARS-CoV-2 infection. Higher viral loads have been recorded in the nose versus the throat, with similar viral loads seen in asymptomatic and symptomatic patients.24 Understanding the biological properties of SARSCoV-2 enabled researchers to develop diagnostics for detection.

DIAGNOSTIC TESTS FOR COVID-19

1. Computed Tomography (CT)

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. The images are analyzed by radiologists to look for abnormal features that can lead to a diagnosis. The imaging features of COVID-19 are diverse and depend on the stage of infection after the onset of symptoms. The most common hallmark features of COVID-19 include bilateral and peripheral ground-glass opacities (areas of hazy opacity) and consolidations of the lungs (fluid or solid material in compressible lung tissue). 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.

COVID-19 infected lung showing Lesions.



Study flowchart followed for confirmed cases of COVID-19 in Wuhan, China by CT scan.

As pneumonia progresses, the functional lung volume decreases. This was likely caused by the swelling of infected lung tissue and filling of alveoli with exudate, leading to a partial loss of lung function. 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.

2. Nucleic Acid Testing

In molecular biology technology, nucleic acid detection methods have developed rapidly and become a revolutionary technology for virus detection. Especially, the method based on polymerase chain reaction (PCR) is characterized by rapid detection, high sensitivity and specificity, which has been regarded as the "gold standard" for virus detection. Several molecular tests which employ non-PCR-based methods, such as isothermal nucleic acid amplification (loop mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification), were developed for the detection of coronavirus RNA.

PCR is an enzymatic method to produce numerous copies of a gene by separating the two strands of the DNA containing the gene segment, marking its location with a primer, and using a DNA polymerase to assemble a copy alongside each segment and continuously copy the copies. Generally, coronavirus RNA is transferred into cDNA by reverse transcription. Afterwards, the PCR is performed and followed by the detection of PCR product through specific detection methods or instruments. Among these, gel visualization and sequencing after PCR are the conventional methods for the detection of corona viruses. However, due to its time-consuming process and high cost, these methods are not commonly used in clinical

samples. Real-time reverse transcriptase-PCR (RT-PCR) detection is currently favored for the detection of coronavirus because of its advantages as a specific, and simple quantitative assay. Moreover, real time RT-PCR is more sensitive than the conventional RT-PCR assay, which help much for the diagnosis in early infection. Therefore, the real-time RT-PCR assay still is a predominant method to be applied for the detection of all kinds of coronaviruses, including SARS-CoV-2.

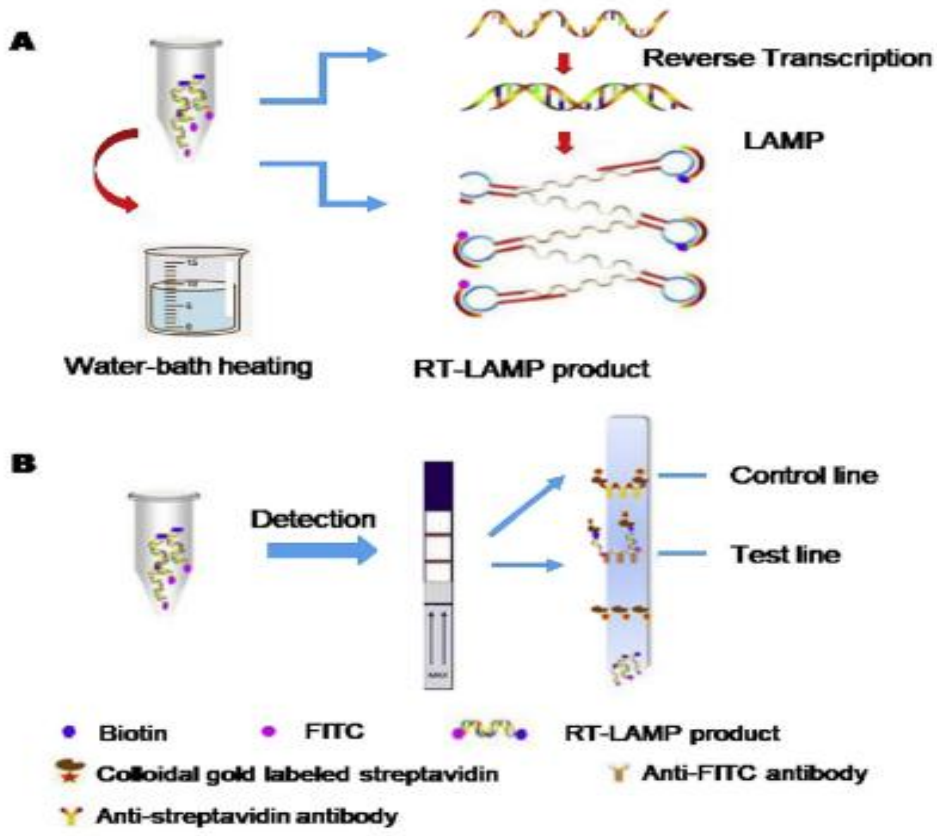


Fig: Schematic illustration of the RT-LAMP-VF Assay. (A) Amplification reaction for RT-LAMP. (B). Detection on visualization strips.

Isothermal nucleic acid amplification-based methods

- **Regular LAMP-based method:** The LAMP assay is rapid and does not require expensive reagents or instruments. Therefore, the application of LAMP test might help to reduce the cost for detection of coronavirus. Gel electrophoresis is commonly used to analyze the amplified products for an endpoint detection. The detection rates and the sensitivity for SARS-CoV in the LAMP assay are similar to those of conventional PCR-based methods. Amplification can be detected as the precipitation of magnesium pyrophosphate or fluorescence dye. This enables the methods to be carried out in real

time by monitoring the turbidity of the pyrophosphate or fluorescence, which has effectively overcome the limitations of the endpoint detection.

- **Sequence-specific LAMP-based method:** If the methods rely on nonspecific signal transduction schemes, such as the fluorescence dyes intercalation into any double-stranded DNA amplicons, or solution turbidity due to the release of pyrophosphates during polymerization, the possibility of unexpected signals derived from primer dimer or non-primer reactions cannot be excluded. A sequence-specific and robust method for monitoring LAMP and other isothermal amplification reactions that can readily separate true signal from nonspecific noise would address this problem.
- **Rolling circle amplification-based methods:** The rolling circle amplification (RCA) has attracted considerable attention in nucleic acid determination. In isothermal conditions, RCA is capable of a 109 -fold signal amplification of each circle within 90 min. An efficient assay for the detection of SARS-CoV by RCA was set up in both liquid and solid phases, and presented preliminary results on a small number of clinical respiratory specimens. The main advantage of RCA is that it can be performed under isothermal conditions with minimal reagents and avoids the generation of false-positive results, which is frequently encountered in PCR-based assays.

MICROARRAY-BASED METHODS

The microarray is a detection method with rapid and high throughput. For this method, the coronavirus RNA will first produce cDNA labeled with specific probes through reverse transcription. Then these labeled cDNAs will be loaded into each well and hybridize with solid-phase oligonucleotides fixed on the microarray, followed by a series of washing steps to remove free DNAs. Finally, the coronavirus RNA can be detected by the detection of specific probes. Due to its superiority, the microarray assay has been widely used in the detection of coronavirus.

3. POINT OF CARE TEST

Abbott ID

The ability to provide (PoC) testing can deliver great improvements to the efficiency and effectiveness of disease management at he individual, local and macro level. For instance, the Abbott ID now is a rapid molecular flu test analysing samples obtained using a nasopharangeal swab to provide diagnosis within 13 minutes. Any PoC test instrumentation ideally needs to be widely available, so it’s only the manufacture of the final products that

needs to be scaled up. Simplicity is also the key, and existing solutions such as the Abott ID now might provide an effective blueprint, being based on minimal sample preparation and constant temperature RNA amplification

Antibody based PoC test

This offers the simplest possible format, being based on lateral flow strips (similar to pregnancy test). 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.

Current treatment strategies for COVID-19

1. Virally targeted inhibitors

Remdesivir, an adenosine analogue that can target the RNA dependent RNA polymerase and block viral RNA synthesis, has been a promising antiviral drug against a wide array of RNA viruses (including SARS/MERS-CoV) infections in cultured cells, mice and nonhuman primate models. Remdesivir and chloroquine have been demonstrated to inhibit SARS-CoV-2 effectively in vitro. Hence, other nucleoside analogues, such as favipiravir, ribavirin and galidesivir, may be potentially clinically applicable against SARS-CoV-2. Chymotrypsin-like (3C-like protease, 3CLpro) and papain-like protease (PLP) are non-structural proteins, which have an essential function for coronaviral replication and can inhibit the host innate immune responses. So 3CLpro inhibitors, such as cinanserin and flavonoids, and PLP inhibitors, such

as diarylheptanoids, are other attractive choices to fight against SARS-CoV-2. ACE2 mediates SARS-CoV-2 entry into the cell as a functional receptor of coronaviruses. So blocking the binding of S protein with ACE2 is also a meaningful strategy against SARS-CoV-2 infection.

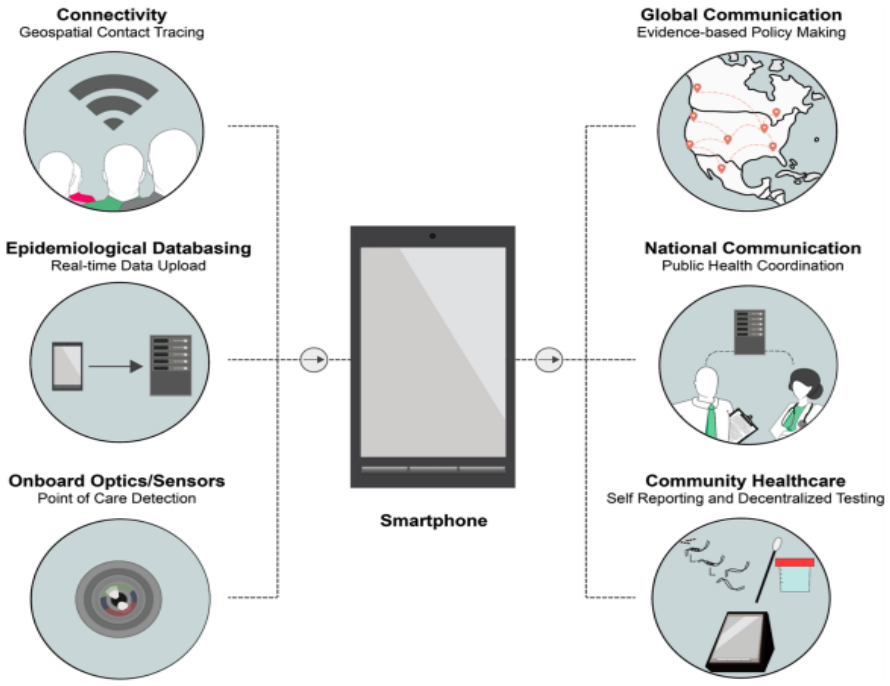
2. Antibody and plasma therapy

The generation of recombinant human monoclonal antibody (mAb) is a fairly straightforward path to neutralize SARS-CoV. CR3022, a SARS coronavirus-specific human monoclonal antibody, can bind potently with the receptor-binding domain (RBD) of SARS-CoV-2 and has the potential to be developed as candidate therapeutics of SARS-CoV-2 infections. Other monoclonal antibodies neutralizing SARS-CoV, such as m396, CR3014, could be an alternative for the treatment of SARS-CoV-2.

3. Vaccines

Effective SARS-CoV-2 vaccines are essential for reducing disease severity, viral shedding and transmission, thus helping to control the coronavirus outbreaks. There are several vaccination strategies against SARS-CoV, MERS-CoV tested in animals, including a live attenuated virus, viral vectors, inactivated virus, subunit vaccines, recombinant DNA, and proteins vaccines. These studies are in progress, but it requires months to years to develop the vaccines for SARS-CoV-2.

Smartphone Surviellance



Controlling epidemics requires extensive surveillance, sharing of epidemiological data, and patient monitoring. 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. 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. The global spread of COVID-19 has been catalyzed by insufficient communication and underreporting.

Countries should track the quantity and results of testing and consider reporting to WHO. Indicators could include the number of SARI/ILI cases reported (compared with previous years in same month/week), the number of patients tested for COVID-19, the number of patients who test positive for COVID-19, the number of tested suspected cases per 100,000 population, and the number of ICU admissions for COVID19.

CONCLUSION

The availability of established diagnostic technologies have enabled researchers to plug-and-play in the design of COVID-19 diagnostics. Such technologies took decades to optimize, but they are now playing an important role in identifying and managing the spread of COVID-19. The rapid identification and sequencing of SARS-CoV-2 has enabled the rapid development of nucleic acid tests. These approaches provide a first line of defense against an outbreak. The combination of diagnostics and smartphones should provide greater communication and surveillance. In conclusion, diagnostics are an important part of the toolbox for dealing with outbreaks because they enable healthcare workers to direct resources and efforts to patients with COVID-19. This process can curb the spread of infectious pathogens and reduce mortality.

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