



AMERICAN JOURNAL OF LIFE SCIENCE AND INNOVATION (AJLSI)

ISSN: 2833-1397 (ONLINE)

VOLUME 1 ISSUE 2 (2022)



PUBLISHED BY: E-PALLI, DELAWARE, USA

Laboratory Diagnosis of Novel Human Coronavirus (SARS-CoV-2) Infections-A Review

Aswathy C Ashok^{1*}, R. Harish¹

Article Information

Received: November 26, 2022

Accepted: November 29, 2022

Published: December 03, 2022

Keywords

*SARS-CoV2, RTPCR,
Immunological Rapid Assay*

ABSTRACT

COVID-19 is a pandemic, highly contagious infectious disease caused by the Severe Acute Respiratory Syndrome Corona virus-2 (SARS-CoV-2). The World Health Organization has declared the ongoing outbreak a global public health emergency. This disease has spread rapidly and affected millions of people worldwide. Currently, there are no specific clinical signs or symptoms of SARS that can be used to differentiate it from other causes of community- or hospital-acquired viral pneumonia. Accurate diagnosis of cases holds the key to managing any pandemic through identification, isolation, and treatment of patients while defining the epidemiology of the pathogen. Because an increasing number of asymptomatic symptomatic individuals must be tested for COVID-19, a safe and efficient screening system is required. The diagnosis of suspected cases is presently confirmed by nucleic acid assays with real-time PCR using respiratory samples. On the other side, serological tests are comparatively easier to perform, but their utility may be limited by their ease of performance and the fact that antibodies appear later in the disease course. This review is aimed at summarizing the currently available information on different methods used for screening and diagnosing COVID-19 infections.

INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a novel virus that caused the first major pandemic disease in the family Coronaviridae. Many coronavirus infections in the past 20 years were not regarded as highly pathogenic to human beings until the outbreaks of SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) (Zhong *et al.*, 2003; Drosten *et al.*, 2003; Fouchier *et al.*, 2003). At the end of 2019, the China Office of the WHO (World Health Organization) reported a cluster of pneumonia cases in Wuhan City, China, and the causative pathogen was identified as Novel Coronavirus (nCoV 2019); the WHO named this disease COVID-19 (Wu *et al.*, 2020; Zhou *et al.*, 2020; Wang *et al.*, 2020).

COVID-19 is an extremely infectious disease. Coronaviruses are enveloped RNA viruses belonging to the Coronaviridae family and the order Nidovirales, which contain approximately 27–32 kilobytes of positive-sense single-stranded RNA. This subfamily consists of four genera: Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and Delta coronavirus, on the basis of their phylogenetic relationships and genomic structures. These subfamilies are broadly distributed and cause infections in humans and other mammals. Alpha and beta coronaviruses infect only mammals. Gamma and delta coronaviruses infect birds, but some of them can also infect mammals. Although the source of the beta coronavirus 2019 SARS Cov-2 is unknown, initial cases have been linked to the South Hunan seafood market. The infected people may have severe symptoms in the respiratory and digestive organs. Like other coronaviruses, the SARS-CoV-2 has at least six

open reading frames (ORFs) and many other accessory genes. There are two open reading frames (ORFs) at the 5' terminal two-thirds of the genome, ORF1 and ORF2. These ORF encode two polypeptides, namely pp1a and pp1ab, which are further cleaved into 11 and 16 proteins, respectively. Nucleocapsid (N), membrane protein (M), envelope protein (E), and spike (S) are among the structural proteins found at 3' terminals. In the case of COVID-19, the spike protein appears to be the primary protein interacting with host cells. Hence, the spike protein is likely the protein to which antibodies are raised, but this is not clear at this time. These viruses also contain some accessory proteins, which aid in virus replication. The S gene aids SARS-Cov-2 in host specificity and receptor binding, and some virion may also contain hemagglutinin esterase (HE) protein. (Cui, J., *et al.*, 2019; Huang C *et al.*, 2020 and Miller *et al.*, 2016) Clinical manifestations of COVID-19 infection include fever and cough as primary clinical manifestations, as well as shortness of breath and myalgia. Some patients have serious complications such as acute respiratory distress syndrome (ARDS) and cytokine storm, which may lead to death. (Malik *et al.*, 2020). It has the capacity for human-to-human transmission. The lack of awareness in hospital infection control and international air travel facilitated the rapid global dissemination of this agent.

The collection of appropriate specimens is very crucial for the detection of most of the infected cases of COVID-19. Nasopharyngeal swabs are typically collected, but we may miss the detection in some cases; therefore, lower respiratory tract specimens such as sputum and bronchoalveolar lavage (BAL) may be an alternative choice. Therefore, there is an urgent need to have an

¹ Department of Biosciences Mar Thoma College, Thiruvalla, Kerala, India

* Corresponding author's e-mail: aswathycashok5@gmail.com

accurate, rapid, readily available, and reliable diagnostic test for SARS-CoV-2 infection. Various immunological, nucleic acid, and amplification diagnostic tests have been developed and are widely available to date. Various integrated point-of-care molecular devices are currently under development, and some are available to provide accurate and fast diagnostic services for SARS-CoV-2 infections. (Loeffelholz *et al.*, 2020).

In view of the present crisis of the COVID-19 pandemic, fast and reliable testing strategies are imperative. In this review, we attempt to learn more about the current diagnostic methods for SARS and Cov-2 infections. The different methods used for screening and sample collection will also be discussed.

Screening and Specimen collection of patients with COVID-19 pneumonia.

Accurate diagnoses of cases hold the key to managing any pandemic through identification, isolation, and treatment of patients while defining the epidemiology of the pathogen. Because an increasing number of asymptomatic symptomatic individuals must be tested for COVID-19, a safe and efficient screening system is required. Presently, no specific clinical signs or symptoms of COVID-19 can be used to differentiate it from other causes of community- or hospital-acquired pneumonia. In order to decide if a patient should be tested, WHO published case definitions for surveillance but encouraged countries to adapt these depending on their local epidemiological situation and other factors. (WHO 2020). A suspect case is defined as (i) a patient with acute respiratory illness (fever and at least one sign or symptom of respiratory disease, such as cough or shortness of breath) and a history of travel to or residence in a location reporting community transmission of COVID-19 disease within 14 days of the onset of symptoms (ii) a patient with severe acute respiratory illness (fever and at least one sign or symptom of respiratory disease, e.g., cough, shortness of breath, and requiring hospitalization) and in the absence of an alternative diagnosis that fully explains the clinical presentation. As per the WHO guidelines, a “probable case” is a suspect case for whom testing for the SARS-CoV-2 is inconclusive or for whom testing could not be performed for any reason. WHO further defines a “contact” as a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case: (i) face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 min. (ii) direct physical contact with a probable or confirmed case. (iii) direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment. For confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample was taken, which led to confirmation. (Venter, M., & Richter, 2020). Specific and real-time diagnostic tests should be performed not

only for the identification of potential cases but also for contacts who need to be quarantined and guided on epidemiological questions around the infection. Selection of the relevant specimen and knowledge of the incubation period, viremia, and shedding period are important criteria in diagnosing individual cases and defining transmissibility to inform the extent of isolation periods for patients. Nucleic acid testing (reverse transcriptase PCR) is recommended for the diagnosis of acute cases. Serological assays have an important role in answering epidemiological questions, including determining the exposure rate and accessing community spreads, but are not relevant for accurate diagnoses of acute cases. Only laboratory confirmation can be used to make an etiological diagnosis and differentiate atypical pneumonia from other causes. Proper collection of samples is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to a negative result.

As per the Center for Disease Control and Prevention (CDC) recommendations, the upper respiratory specimen should be collected for RT-PCR-based testing of COVID-19, and especially the nasopharyngeal exudate is the preferred choice (CDC, 2020). Within a week from the onset of symptoms, patients with COVID-19 usually possess high viral loads in their upper and lower respiratory tracts. (Zhou *et al.*, 2020). A nasopharyngeal swab and/or an oropharyngeal swab are often recommended for screening or diagnosing early infections. Nasopharyngeal swabs usually reach the correct area to be tested in the nasal cavity (Wang *et al.*, 2020) reported that oropharyngeal swabs were used much more frequently than nasal swabs in China during the current COVID-19 outbreak; however, the SARS-CoV-2 RNA was detected in only 32% of oropharyngeal swabs, which was significantly lower than the level in nasal swabs (63%). for the late detection and monitoring of patients with severe COVID-19 pneumonia. Ideally, sputum sampling or bronchoalveolar lavage (BAL) has been used for collecting lower respiratory tract specimens as they have yielded the highest viral loads for the diagnosis of COVID-19 (Li *et al.*, 2020; Yu *et al.*, 2020). A recent study revealed that samples of bronchoalveolar lavage fluid yielded the highest SARS-CoV-2 RNA rate, although this study did not compare or evaluate results from nasopharyngeal swabs (Wang *et al.*, 2020). Patients who present with severe pneumonia and acute respiratory distress syndrome may require emergent intubation as well as respiratory isolation in a negative-pressure room. If possible, a lower respiratory tract sputum specimen should be collected during the intubation procedure. Alternatively, sputum and/or bronchoalveolar lavage fluid specimens may be collected after intubation (Pan *et al.*, 2020). Collecting a respiratory swab specimen may carry a theoretical risk of transmitting SARS-CoV-2, particularly if chances of airborne transmission are demonstrated. Currently, biosafety level (BSL)-2 conditions are recommended for the handling of specimens for

molecular testing. While collecting specimens, health care professionals should follow WHO infection prevention and control guidelines and wear personal protective equipment (PPE) such as gloves, gowns, eye protection, and N95 masks (WHO 2020). All specimens collected for laboratory investigations are regarded as potentially infectious.

Testing of specimens from multiple sites (e.g., upper and lower respiratory tracts) may improve the sensitivity of the RT-PCR and reduce false-negative test results, especially during the second week of illness. For asymptomatic patients and patients with mild symptoms, the collection of both nasopharyngeal swabs and oropharyngeal swabs is recommended; these should be placed together in the same viral transport medium (VTM) to increase the sensitivity (WHO 2020, CDC 2020). Additional clinical specimens can be collected as the COVID-19 virus has been detected in blood, urine, and stool. In the case of deceased patients, the collection of autopsy material, including lung tissue, is also considered. (Kaijin *et al.*, 2020). As serological assays become available retrospectively in recovered patients, paired serum (acute and convalescent) can be useful in defining cases (Zhang *et al.*, 2020; ShiX *et al.*, 2005; Ding Y. *et al.*, 2004). Several studies reported the detection of higher viral loads in older patients.

However, the viral load did not positively correlate with disease severity (Tsang *et al.*, 2020; Pung *et al.*, 2020; Wang *et al.*, 2020). Since SARS-CoV-2 and most other respiratory viruses are RNA-based, care should be taken to select extraction and inactivation protocols that will not damage RNA. A recent study suggests that heat inactivation at 56 °C for 30 min may result in false negatives for samples with low viral loads (Pan *et al.*, 2020). Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation is essential.

Specimens that can be delivered to the laboratory can be stored and shipped at 2–8 °C. When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium (VTM) is strongly recommended in the laboratory biosafety guidance related to the novel coronavirus (2019-nCoV). (Guidance on regulations for the transport of infectious substances, 2019–2020) The primary container should be sealed with a screw cap. The container should be made of plastic that has a low risk of breakage. Patient information should be recorded on the specimen container using two or more identifiers (e.g., name, patient number), along with the necessary information for testing requests.

The outer surface of the primary container should be disinfected using appropriate disinfectants, such as 70% ethanol. The container should be packed in a zipper bag and placed in a secondary container before transportation. The secondary container should be sealed and shock-resistant, and it should be labelled to indicate that it contains infectious substances. When transporting specimens to a laboratory within the same institution, the specimens should be transported in person; the pneumatic tube system should not be used. A separate route should be used for transportation.

The personnel transporting the specimen should be trained in spill decontamination procedures in case of specimen leakage. Specimens may be frozen to -20°C or, ideally, -70°C and shipped on dry ice if further delays are expected. It is important to avoid repeated freezing and thawing of specimens (Kwon *et al.*, 2020; Korea Center for Disease Control and Prevention, 2020; CDC, 2020). WHO documents a summary of the optimum sample collection procedures and storage, which are similar to those for influenza. The specimen collection and storage temperature protocol to investigate emerging acute respiratory diseases is summarized in Table 1.

Table 1: The specimen collection and storage protocol to investigate non-seasonal influenza and other emerging acute respiratory diseases

Specimen types	Collection material	Storage temperature
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs	2-8°C
Sputum	Sterile container	2-8°C
Bronchoalveolar lavage	Sterile container	2-8°C
Nasopharyngeal or nasal aspirates or washes	Sterile container	2-8°C
Tissue from biopsy or autopsy, including lung tissue	Sterile container with saline or VTM	2-8°C
Serum	Serum separator tubes	2-8°C
Whole blood	Collection tube	2-8°C
Stool	Stool container	2-8°C
Urine	Urine collection container	2-8°C

Surveillance and Sampling Strategies

Individual case definition mechanisms (2.1), as mentioned above, help choose whether a patient should be tested or not. However, a pandemic like COVID-19 necessitates robust surveillance systems at local and global levels, which are required to strengthen the

effective implementation of control measures. The aim of these surveillance systems is to limit the spread of disease, enable public health authorities to manage the risk, and thereby enable economic and social activity to resume to the extent possible. Surveillance is also necessary to predict the longer-term trends of COVID-19

transmission and the changes ahead (WHO 2020). A “case sample” is considered a subset of individuals from a larger population. Sampling is simply stated as selecting a portion of the infected population that will actually collect evidence from the research area under surveillance. Samples are used to make inferences about populations. (Landreneau, K. J., *et al.*, 2009).

There are two types of sampling methods employed in epidemiological surveillance:

Probability Sampling

This includes some form of random selection in choosing the elements. Greater confidence can be placed in the representativeness of probability samples. This type of sampling involves a selection process in which each element in the population has an equal and independent chance of being selected.

- Simple random sampling: in a simple random sample, every member of the population has an equal chance of being selected.
- Stratified random sampling: this sampling method is appropriate when the population has mixed characteristics (like age, demography, etc.) and you want to ensure that every characteristic is proportionally represented in the sample.
- Cluster sampling: cluster sampling also involves dividing the population into subgroups, but each subgroup should have similar characteristics to the whole sample. Instead of sampling individuals from each subgroup, entire subgroups are randomly selected.
- Systematic Sampling: This is similar to simple random sampling, but it is usually slightly easier to conduct. Every member of the population is listed with a number, but instead of randomly generating numbers, individuals are chosen at regular intervals for sampling.

Non-probability Sampling

The elements that make up the sample are selected by nonrandom methods. This type of sampling is less likely than probability sampling to produce representative samples. Even though this is true, researchers can and do use non-probability samples. The three main methods are:

- Convenience Sampling: A convenience sample includes the individuals who happen to be most accessible to the researcher.
 - Quota sampling is primarily motivated by ease of access. Instead of the researcher choosing participants and directly contacting them, people volunteer themselves (e.g., by responding to a public online survey).
 - Purposive Sampling: This type of sampling involves the researcher using their judgement to select a sample that is most useful for the purposes of the research.
- Any COVID-19 surveillance system placed should be geographically comprehensive and include all people and communities at risk. Surveillance for vulnerable or high-risk populations should be enhanced. This will require a combination of surveillance systems, including

contact tracing, in the entire health care system, at the community level, as well as in closed residential settings and for vulnerable groups. Surveillance at the primary care level is needed to detect cases and clusters in the community. Where possible, testing facilities are made available at primary care clinics. A complementary option is to establish dedicated COVID-19 community testing facilities. Patients with probable and confirmed COVID-19 cases are notified within 24 hours of identification. Fast data reporting and analysis are critical to detecting new cases and clusters. Therefore, only the minimum number of data variables are to be collected (e.g., age, sex, date of illness onset, date of sample taken, test result, location of testing site, etc.). Data reporting to local or national public health authorities is done on a daily basis. Patients with probable or confirmed COVID-19 diagnoses in hospitals are notified within 24 hours of identification. All COVID-19 deaths are reported within 24 hours of the death. The minimum essential data from hospital settings includes: age, sex/ gender, and place of residence; date of onset; date of sample collection; date of admission; laboratory test result; severity on admission: admitted to the intensive care unit (ICU); treated with ventilation, if the case is a health care worker; outcome (discharge or death); etc. Existing sentinel surveillance of influenza-like illness (ILI) or acute respiratory infections (ARI) is useful to monitor trends in community transmission of the COVID-19 virus and to ensure that other priority respiratory diseases are being detected. Integration of COVID-19 with the Global Influenza Surveillance and Response System (GISRS) is described in operational considerations for COVID-19 surveillance using GISRS. Virologic sentinel surveillance of COVID-19 is conducted using clinical specimens obtained through sentinel surveillance of ILI, ARI, and SARI (Severe Acute Respiratory Infection). Integrated epidemiological and virological surveillance will play a significant role in monitoring the spread and evolution of COVID-19 virus, understanding the cocirculation of COVID-19 virus with influenza and other respiratory viruses, and subsequent interpretation of respiratory epidemiological and disease observations in relation to COVID-19, as well as supporting the update of diagnostic tests. Infections in health workers should, at a minimum, be systematically integrated into the national surveillance system. Dedicated enhanced surveillance for some high-risk groups is necessary to ensure the prompt detection of cases and clusters, faster than through primary care or hospital-based surveillance. People who live in closed environments, such as prisons, or residential facilities, such as retirement communities or care homes for persons with disabilities, can be especially vulnerable because they may not be able to seek help themselves. Vulnerable groups may also live in settings where the probability of transmission is higher than in the general population or have health conditions or predisposing factors that increase their risk of severe illness. Enhanced surveillance includes the use of active case finding, such as

through daily screening of signs and symptoms, including daily temperature monitoring, and daily zero reporting for all individuals in high-risk groups under surveillance. While surveillance systems will typically capture the number of COVID-19 cases, it is also important to collect information on the total number of laboratory tests conducted for the COVID-19 virus. Data on the number of tests conducted for SARS-CoV-2 are collected from all relevant laboratories. Knowing the testing denominator can indicate the level of surveillance activity, and the proportion of positive tests can indicate the intensity of transmission among symptomatic individuals. Presently, reverse transcriptase polymerase chain reaction (RT-PCR) testing (explained in 2.3) is the most common and reliable laboratory diagnostic method. If other diagnostic methods are used, the number of tests conducted and cases confirmed by different laboratory diagnostic methods need to be recorded.

It is widely accepted that a large fraction of COVID-19 cases goes undetected. However, this is subject to significant ascertainment bias because tests are typically ordered only from symptomatic cases, whereas a large proportion of infected people may show little to no symptoms (Mizumoto *et al.*, 2020). Non-symptomatic infections can still shed the SARS-CoV-2 virus and are therefore detectable by RT-PCR-based tests. It is therefore possible to test randomly selected individuals to estimate the true disease prevalence in a population. Recent technical advances have enabled high-throughput PCR, in which multiple samples are pooled into one tube. Combining probes from several individuals and testing them together reduces the total amount of testing needed. This method is known as “pooling” or “group testing.” The main idea is that when samples from several people are mixed together and tested, the test will report negative when everyone is healthy and positive when at least one is positive (Abdallhamid *et al.*, 2020). The other ideas include using ten-fold fewer tests (Verdun *et al.*, 2021) and clearing 20 times the number of people from isolation with the same number of tests (Gollier and Gossner, 2020).

However, their efficiency is highly dependent on the frequency of positive samples, which varies significantly across regions and even within regions as testing criteria and conditions change. Two possible optimized pooling strategies are currently employed for diagnostic SARS-CoV-2 testing on large scales; both address dynamic conditions. In the first, an estimate of the target frequency determines the initial pool size, and any subsequent pools found positive are re-pooled at half size and tested again. The second method is a simpler approach of optimized one-time pooling followed by individual tests on positive pools. These strategies are convenient, and they offer a significant reduction in the number of materials, equipment, and time needed to test large numbers of samples. (Shani *et al.*, 2020). On the other hand, a pool testing strategy could potentially increase worldwide testing capacity many times over, thus boosting a

country’s capacity to test mildly to asymptotically affected individuals. This strategy proposes that instead of individually testing patients with low clinical suspicion of SARS-CoV-2 infections, samples are pooled together in what is called a “minipool” and then tested together, running a single RT-PCR for all the unified samples. Preliminary results showed that there is no dilution and no decrease in test sensitivity when minipools of five samples each are used. Since the RT-PCR looks directly at the viral RNA, a negative result in a pool test is reliable. Thus, the infection was discarded in all the patients included in the pooled sample.

Nucleic Acid Amplification Assay

SARS-CoV-2 is a single-stranded, positive-sense RNA virus. The availability of sequence data has facilitated the design of primers and probes needed for the development of SARS-CoV-2-specific testing. Routine confirmation of cases of COVID-19 is based on the detection of unique sequences of virus RNA by real-time reverse transcription polymerase chain reaction (RT-PCR), with confirmation by nucleic acid sequencing when necessary. The majority of molecular diagnostic tests have utilized real-time RT-PCR technology targeting different SARS-CoV-2 genomic regions, including the ORF1b or ORF8 regions and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope (E) genes.

The most widely used method of COVID-19 diagnostics is a reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay to detect the presence of SARS-CoV-2 RNA in patient samples, typically nasopharyngeal swabs. RNA extraction is a major bottleneck in current COVID-19 testing. (Shen *et al.*, 2020; Smyrlaki *et al.*, 2020). Because of its high sensitivity and specificity, polymerase chain reaction (PCR) is regarded as the gold standard test for the molecular diagnosis of viral and bacterial infections. Isothermal nucleic acid amplification, including transcription-mediated amplification and CRISPR-based methodologies, is considered a promising alternative assay due to its fundamental advantage in quick procedure time at constant temperature without thermocycler operations. As such, real-time reverse transcriptase-PCR (RT-PCR) is of great interest today for the detection of SARS-CoV-2 due to its benefits as a specific and simple qualitative assay. Furthermore, real-time RT-PCR has sufficient sensitivity to aid us in early infection diagnosis. Therefore, the “criterion-referenced” real-time RT-PCR assay can be considered as the main method to be applied to detect the causative agent of COVID-19, i.e., SARS-CoV-2 (Carter *et al.*, 2020; Shen *et al.*, 2020; Wan *et al.*, 2016; Noh *et al.*, 2017; etc.).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

In acute respiratory infections, RT-PCR is routinely used to detect causative viruses from respiratory secretions in nucleic acid testing assays. The real-time reverse

transcription (PCR) method is one of the best and most accurate laboratory methods for detecting, tracking, and studying the coronavirus. Real-time RT-PCR is a method by which the presence of specific target genetic material can be detected (Sethuraman *et al.*, 2020). This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the viral RNA genome and the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent dye or a sequence-specific DNA probe labelled with a fluorescent molecule until the viral cDNA can be detected. Coronaviruses have a number of molecular targets within their positive-sense, single-stranded RNA genome that can be used for PCR assays (Corman *et al.*, 2020).

These include genes encoding structural proteins, including envelope glycoproteins spike (S), envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N) (Chan *et al.*, 2020). In addition to the genes that encode structural proteins, there are species-specific accessory genes that are required for viral replication, like RNA-dependent RNA polymerase (RdRp), hemagglutinin esterase (HE), and open reading frames ORF1a and ORF1b (Corman *et al.*, 2020; Lan *et al.*, 2020). The assay includes at least two molecular targets to avoid potential cross-reaction with other endemic coronaviruses as well as potential genetic drift of SARS-CoV-2. In the United States, the CDC recommends two nucleocapsid protein targets (N1 and N2) (Holshue *et al.*, 2020), while the WHO recommends first-line screening with an E gene assay followed by a confirmatory assay using the RdRp gene (Corman *et al.*, 2020). RT-PCR has traditionally been carried out as a one-step or two-step procedure. One-step real-time RT-PCR uses a single tube containing the necessary primers to run the entire RT-PCR reaction. Two-step real-time RT-PCR involves more than one tube to run the separate reverse transcription and amplification reactions but offers greater flexibility and higher sensitivity than the one-step procedure. (VanGuilder, H. D., *et al.*, 2008; Wong *et al.*, 2005).

Positive test results from a single sample must be confirmed by a repeat test detecting a different region of the SARS-CoV-2 genome on the same sample. If possible, another repeat sample should also be tested to exclude false positive results due to amplicon carryover. Since the viral load in nasopharyngeal aspirate usually peaks on the 10th day after the onset of symptoms, suspected SARS cases must have the tests repeated as the disease evolves to avoid false-negative results. Stool specimens should be sent for testing on a regular basis, as a high percentage of patients develop diarrhoea and shed virus during the second week of illness. Viral load determination of nasopharyngeal specimens or serum upon presentation might have clinical value as it is an important prognostic factor. Any treatment regimen would benefit from long-term monitoring of viral load.

(Cheng *et al.*, 2004; Peiris *et al.*, 2003; Chan K. *et al.*, 2004; Chu *et al.*, 2020; etc.). Apart from sensitivity issues, RT-PCR has some other drawbacks, such as possible biological safety hazards that may occur during transport and sample processing, nucleic acid extraction, and the requirement of sophisticated laboratory equipment like biosafety cabinets. Technical expertise, along with sample transportation, which is inevitable, makes the overall process time-consuming. All these drawbacks could make the process less useful in case of a health emergency or global outbreak situation. Moreover, in PCR, we are able to detect not only the target virus, but it can also perform codetection of several other respiratory viruses, which leads to an increase in false positive or negative results (Cho *et al.*, 2014).

Isothermal nucleic acid amplification

Isothermal nucleic acid amplification is an alternative strategy that allows amplification at a constant temperature and eliminates the need for a thermal cycler. Therefore, several methods based on this principle have been developed. Isothermal amplification techniques are conducted at a single temperature and do not need specialized laboratory equipment to provide similar analytical sensitivities to PCR. isothermal amplification techniques can be multiplexed during the amplification or readout stage.

This is done by using polymeric beads encoded with unique optical signatures like organic fluorescent molecules for each gene. Multiplexing increases the amount of information gained from a single test and improves clinical sensitivity and specificity. Loop-Mediated Isothermal Amplification (LAMP) is a relatively new molecular amplification point-of-care technique that is widely used for COVID-19 diagnosis. The technique is based on the synthesis of target DNA at a constant temperature of 60–65°C using a specially designed primer and DNA polymerase that has strand displacement activity instead of heat denaturation as in other PCR techniques.

This novel technique can amplify any genomic material with high efficiency and in a shorter amount of time. RT-LAMP has been developed as a rapid and cost-effective testing alternative for SARS-CoV-2. It requires a set of four primers specific for the target gene or region to enhance the sensitivity and combines LAMP with a reverse transcription step to allow for the detection of RNA. Photometry can be used to detect the amplification product by measuring the turbidity caused by magnesium pyrophosphate precipitate in the solution as a byproduct of amplification.

The reaction can be followed in real time either by measuring the turbidity or by fluorescence using intercalating dyes. Since real-time RT-LAMP diagnostic testing requires only heating and visual inspection, its simplicity and sensitivity make it a promising candidate for virus detection. The RT-LAMP test uses reverse transcriptase to convert the viral RNA to cDNA, which

is subsequently amplified by the DNA-dependent DNA polymerase for rapid colorimetric detection with a DNA-binding dye.

LAMP has been shown to be effective at detecting viral RNA in cell lysates at levels of approximately 480 RNA copies without interference, providing an alternative to RT-PCR for rapid and simple detection of SARS-CoV-2 RNA. (Notomi *et al.*, 2000; Thai *et al.*, 2004). Transcription-Mediated Amplification (TMA) is a patented single-tube, isothermal amplification technology modelled after retroviral replication that can be used to amplify specific regions of either RNA or DNA much more efficiently than RT-PCR. It uses a retroviral reverse transcriptase and T7 RNA polymerase and has been used for the detection of nucleic acids from multiple pathogens.

The initial step involves hybridization of the viral RNA target to a specific capture probe and an additional oligonucleotide containing a T7 promoter primer, which are captured onto magnetic microparticles. Then, the captured RNA target hybridized to the T7 promoter primer is reverse transcribed into a complementary cDNA. The RNase activity of the reverse transcriptase subsequently degrades the target RNA strand from the hybrid RNA cDNA duplex, leaving a single-stranded cDNA, which includes the T7 promoter. An additional primer is used to generate double-stranded DNA, which is subsequently transcribed into RNA amplicons by T7 RNA polymerase. These new RNA amplicons then reenter the TMA process, allowing this exponential amplification to generate billions of RNA amplicons. The detection process involves the use of single-stranded nucleic acid pyrotechnics that hybridize specifically to the RNA amplicon in real time. Each torch is conjugated to a fluorophore and a quencher. When the torch hybridizes with the RNA amplicon, the fluorophore is able to emit a signal upon excitation. (Kacian *et al.*, 1999) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequences found in prokaryotic organisms, such as bacteria. These sequences can be recognized and cut by a set of bacterial enzymes called CRISPR-associated enzymes. Certain enzymes in these families can be programmed to target and cut viral RNA sequences.

The CRISPR-based methods do not require complex instrumentation and can be read using paper strips to detect the presence of the SARS-CoV-2 virus without loss of sensitivity or specificity. These tests are both low-cost and can be performed in as little as an hour. These tests have great potential for point-of-care diagnosis. (Zhang *et al.*, 2020; Broughton *et al.*, 2020). The rolling circle amplification (RCA) method 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 minutes. An efficient assay for the detection of SARS-CoV-2 by RCA has been set up in both liquid and solid phases and has yielded 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 are frequently encountered in PCR-based assays. (Chapin *et al.*, 2011, Xu *et al.*, 2019, Wang *et al.*, 2005, etc.).

Cartridge Based Nucleic Acid Amplification test (CB-NAAT) and True NAAT

Unlike traditional RT-PCR tests, the sample preparation in CB-NAAT tests is automated, and the results are available within half an hour. The test uses nose- or throat-swab samples, which are collected from patients and dipped in a solution that inactivates the virus. A few drops of the solution are then placed on a cartridge. On inserting this cartridge into a machine, a preprogrammed reaction is initiated, which extracts the nucleic acids or the genetic material from the samples. This has to be followed by RT-PCR. The purified nucleic acid is added into a microtube containing freeze-dried RT-PCR reagents and allowed to stand for a minute, then applied to a microchip and then inserted into another machine, where the reverse transcription and PCR take place. The advantage of this test is that it is quick and portable. This allows the easy setup of mobile testing centers or kiosks in containment zones instead of having to transport samples to labs. True NAT is an indigenously developed, portable version of CB-NAAT, also known as the GeneXpert test. Both of these tests were originally designed to screen for tuberculosis.

IMMUNOLOGICAL ASSAYS

Immunological tests measure the antibodies generated by the host body's immune response against the virus infection (antibody test) or the proteins of COVID-19 virus present in the respiratory specimens (antigen test). When a virus enters the human body, it triggers an immune response that results in the production of an antibody against the virus; detecting such an antibody in an infected person is extremely useful regardless of whether the person has symptoms. Antibody tests are blood-based tests that can be used to identify whether people have been exposed to a particular pathogen. The serum includes antibodies to specific components of pathogens, called antigens.

These antigens are recognized by the immune system as foreign and are targeted by the immune response. These types of tests are often used in viral infections to see if the patient has an immune response to a pathogen of interest, such as SARS-CoV-2. The role of serological assays to detect IgG, IgA, or IgM anti-SARSCoV-2 antibodies in serum, plasma, or capillary blood provides a clear picture of the outbreak size in each country and helps to assess the degree of immunization. (Okba *et al.*, 2020). Serological testing for COVID-19 is particularly attractive because of the relatively short time to diagnosis and the ability to test for an active immune response against the virus. Serological tests have variable sensitivity and specificity. Research has demonstrated that the spike

(S) and nucleocapsid (N) proteins are the primary viral antigens against which antibodies are raised. (Chan *et al.*, 2009; Kumar *et al.*, 2020). These antigens are the most commonly used in serological tests. During infection, several types of antibodies are raised against the virus. IgM antibodies emerge first, after 5 days of post-symptom onset. IgG antibodies typically emerge after 10 days of post-symptom onset. Many serology tests detect both IgG and IgM simultaneously, which increases the specificity of the test. IgA antibodies may also increase during infection and are typically found in mucous. While serological tests are now widely available, the correlates of immunity are still poorly understood.

The presence of antibodies only indicates a previous SARS-CoV-2 infection. The results of serological tests can then be used to estimate the true spread of the virus through a population, even if individuals were asymptomatic or were never diagnosed. The presence of antibodies does not indicate that an individual is protected from reinfection since there is limited understanding of the levels and persistence of antibodies necessary for protective immunity. Therefore, serological tests cannot inform an individual of their immunity to reinfection (Yu *et al.*, 2020). However, the test results may also help in choosing convalescent plasma, which can be used as a promising treatment option for COVID-19-infected individuals.

Antibody Tests

The determination of SARS-CoV-2 exposure relies largely on the detection of either IgM or IgG antibodies that are specific for various viral antigens, including the spike glycoprotein (S1 and S2 subunits) and nucleocapsid protein. The methodology for these determinations includes the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and specific luminescent immunoassays. Each of these formats brings advantages (speed, multiplexing, automation) and disadvantages (trained personnel, dedicated laboratory requirements, etc.). Rapid antigen tests, which use antibodies to detect the presence of viral antigen(s) in serological samples, are complementary to these. Major diagnostic companies are currently focusing on the development of high-throughput serology tests.

- Enzyme-linked immunosorbent assays (ELISA): a faster serological test performed in a laboratory that provides a readout of antigen-antibody interactions. Essentially, patient antibodies are “sandwiched” between the viral protein of interest and reporter antibodies so that any active patient antibodies are detected. A serological assay was performed using an ELISA kit that was developed for detecting IgM or IgG antibodies against the N proteins of SARS-CoV-2. For IgM detection, ELISA plates were previously coated with mouse anti-human antibodies. This test can be qualitative or quantitative and is generally a lab-based test that is obtainable within a few hours. These tests usually use whole blood, plasma, or

serum samples from patients. The test relies on a plate that is coated with a viral protein of interest, such as spike protein. Patient samples are then incubated with the protein, and if the patient has antibodies to the viral protein, they bind together. The bound antibody-protein complex can then be detected with another wash of antibodies that produce a colored or fluorescent-based readout. (Liu *et al.*, 2020). ELISA is speedy, has the ability to test multiple samples, and is adaptable to automation for increased throughput, but can be variable in sensitivity and is suitable for point-of-care determinations.

- Lateral flow assays (LFAs): Lateral flow assays (LFAs), also called rapid diagnostic tests (RDTs), display a colorimetric, qualitative readout of the presence of antibodies. These are often used in point-of-care settings. The patient sample is passed through a membrane on which the target antigen is anchored. If the sample contains antibodies specific to that antigen, they form a complex that results in a colored band on the strip. These are similar to pregnancy tests. This is facilitated by a recombinant antigen present on immunochromatographic paper, on which the test sera are applied, and antigen antibody binding is detected visually by a color change on a membrane. The results can be obtained within 15–20 minutes. The test is inexpensive and requires no trained personnel, but it provides only qualitative results. When used in conjunction with rapid antigen tests, where anti-SARS-CoV2 antibodies are used in place of immobilized viral antigen, they allow for a more direct assessment of ongoing infection.

- Neutralization assays: determine an antibody’s ability to prevent virus infection of cultured cells and the cytopathic effects of viral replication. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time to results for neutralization assays is typically 3–5 days, but recent advances have reduced this to hours. This type of testing requires cell culture facilities, and in the case of the SARS coronavirus, Biosafety Level 3 (BSL3) laboratories are required. Despite these limitations, the determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and in the long term for vaccine development.

- Luminescent immunoassay: This test shows whether a patient has antibodies to a pathogen by displaying a fluorescent signal when patient antibodies interact with virus proteins. Luminescent immunoassays comprise methods that lower the limits of detection for antibody-based reagents. Generally, they involve chemiluminescence and fluorescence. For SARS-CoV-2, two-step chemiluminescent immunoassays for the detection of IgG and IgM SARS-CoV-2 antibodies in human serum or plasma have been demonstrated. Samples react with paramagnetic microparticles coated

with SARS-CoV-2-specific antigens (recombinant N- and S-proteins), and alkaline phosphate-labeled anti-human IgG or IgM monoclonal antibodies are added to the reaction mixture, resulting in a chemiluminescent emission, measured as relative light units (RLU) by a photomultiplier built into the system. After 25 minutes, the first results were generated (Nuccetelli *et al.*, 2020)

Antigen Detection Assays

Antigen detection with monoclonal antibodies or monospecific polyclonal antibodies against the protein was found to be a sensitive and specific test for the diagnosis of SARS (Kumar *et al.*, 2020). In a large study with sera collected from 317 SARS patients at different time points of illness, the detection of SARS-N antigen was performed using a panel of three monoclonal antibodies. Over 80% of SARS cases were discovered within the first 7 days of illness. As serum antibody levels started to rise at day 7, the sensitivity of the serum antigen assay progressively decreased to 0% at day 21 (Chan *et al.*, 2004, Hsueh *et al.*, 2003).

RAPID ASSAY METHODS

Rapid tests are non-automated, primarily qualitative (but also quantitative in some cases), and used for in vitro diagnostics. These tests can provide results within 10–30 min, so their results are considered instant as compared to the molecular tests, which generally take 4–6 h. Moreover, these tests are user-friendly, so they won't require any extensive training or expertise to operate and can be used either in a hospital environment or in the field without any difficulty. The manifestation of the COVID-19 infection is highly nonspecific, including respiratory symptoms such as fever, cough, dyspnea, and viral pneumonia (Huang *et al.*, 2020). Thus, extensive diagnostic tests specific to this infection are urgently required to confirm suspected cases, screen patients, and conduct virus surveillance. Rapid tests are used to diagnose patients without sending samples to centralized facilities, thereby enabling communities without laboratory infrastructure to detect infected patients. In this, a point-of-care (PoC) device, i.e., a rapid, robust, and cost-efficient device that can be used onsite and, in the field, and which does not necessarily require a trained technician to operate (Nguyen *et al.*, 2018),

LAMP assays (explained in 2.3.2) in PoC devices have high specificity and sensitivity and are simple to perform; hence, soon after their initial development, they became an enormously popular isothermal amplification method in molecular biology, with applications in pathogen detection. LAMP uses strand-displacement polymerases instead of heat denaturation to generate a single-stranded template; hence, it has the advantage. LAMP technology is proven to be more stable and more sensitive in detection compared to PCR (Francois *et al.*, 2011). Lateral flow antigen detection (explained in 2.4.1) for SARS-CoV-2 is another point-of-care approach under development for diagnosing COVID-19. In commercial lateral flow

assays, a paper-like membrane strip is coated with gold nanoparticle-antibody conjugate, and capture antibodies are used. These assays have previously demonstrated reliable clinical sensitivity (57%), specificity (100%), and accuracy (69) for IgM and 81%, 100%, and 86% for IgG, respectively. A test that detected both IgM and IgG yielded a clinical sensitivity of 82% (Xiang *et al.*, 2020). However, lateral flow assays do not directly confirm virus presence; instead, they provide serological evidence of recent infections (Li *et al.*, 2020). Microarray assays have been used for rapid, high-throughput detection of SARS-CoV-2 nucleic acids. They rely on the generation of cDNA from viral RNA using reverse transcription and the subsequent labelling of cDNA with specific probes loaded into the wells of microarray trays. The microarray assay has proven useful in identifying mutations associated with SARS-CoV-2 and has been used to detect up to 24 single nucleotide polymorphisms (SNP) associated with mutations in the spike (S) gene of SARS-CoV-2 with 100% accuracy. The ability to detect different emergent strains of SARS-CoV-2 may become necessary as the COVID-19 pandemic evolves, and microarray assays provide a platform for rapid detection of those strains as a result of mutational variations. A next-generation shotgun metagenomics sequencing platform has been developed by Illumina with the ability not only to detect the presence of multiple strains of coronaviruses but also to comprehensively examine multiple pathogenic organisms present in a complex sample.

Chest CT images from patients with COVID-19 typically demonstrate bilateral, peripheral ground glass opacities. Because this chest CT imaging pattern is nonspecific and overlaps with other infections, the diagnostic value of chest CT imaging for COVID-19 may be low and dependent upon other interpretations. Given the variability in chest imaging findings, a chest radiograph or CT alone is not recommended for the diagnosis of COVID-19. The American College of Radiology also does not recommend CT for screening or as a first-line test for the diagnosis of COVID-19. Lymphopenia is the most common laboratory finding in COVID-19 and is found in as many as 83% of hospitalized patients. (Huang *et al.*, 2020) Lymphopenia, neutrophilia, elevated serum alanine aminotransferase and aspartate aminotransferase levels, elevated lactate dehydrogenase, high CRP, and high ferritin levels may be associated with greater illness severity (Chen *et al.*, 2020). Patients with critical illness had high plasma levels of inflammatory markers, suggesting potential immune dysregulation (Wang *et al.*, 2020).

This review summarizes various diagnostic methods used for the identification of COVID-19 infections. While RT-PCR has been the dominant technique for detection of viral RNA, other nucleic acid assays, including isothermal amplification assays, hybridization microarray assays, amplicon-based metagenomics sequencing, and the cutting-edge CRISPR-related technologies, are also under development or have resulted in approved tests. A comparative account of different diagnostic methods

Table 1: A comparative account of different diagnostic methods used for detection of COVID-19

Method	Working Principle	Advantage	Time Required	Disadvantage
Next generation sequencing (NGS)	Wholegenome sequencing,shotgun metagenomics etc.	Highly sensitive and specific, could provide all related information; can identify novel strain. Helps to examine multiple pathogenic organisms present in a complex sample.	1-2 days	Require high expertise & cost. Highly sophisticated Lab required.
RT-PCR	Specific primer-probe based detection	Fast results, higher sensitivity, well established methodology in viral diagnostics.	3-4 hrs.	High cost due to expensive consumables. Expensive lab equipment. RNA extraction is highly tedious and sensitive.
Isothermal nucleic acid amplification	Synthesis of target DNA at constant temperature of 60 -65°C using specially designed primer	No thermal cycler required. Can be easily detected by color change or turbidity	1-2 hrs.	Too sensitive, highly prone to false positive results due to carry-over or cross contamination
LAMP	More than two sets of specific primers pair-based detection.	Highly repeatable and accurate. No thermal cycler required	1 hr.	Primer designing is complex
CB NAAT/ True NAAT	Cartridge Based Nucleic Acid Amplification and detection	Automated sample preparation, pre-programmed reaction. Can be used in a PoC devices	15–30 min	
CRISPR based methods	Enzymes programmed to target and cut viral RNA sequences	low-cost, do not require complex instrumentation, can be read using paper strips.	15–30 min	Not properly standardized for SARS Cov2 detection
Serological (traditional)	Antigen/Antibodies IgG/ IgM/ELISA etc.	Sensitive and specific. useful for choosing the convalescent plasma therapy.	4-6 hrs	Samples taken after 3-4 days of infection.
Rapid Serological (traditional)	Antigen/Antibodies IgG/IgM	Convenient, can be used in a PoC devices	15-30 min	Samples taken after 3-4 days of infection.
Lateral flow assay	colorimetric, qualitative detection of the presence of antibodies	Inexpensive and convenient, can be used in a PoC devices	10-15 min	Nature of the sample affects capillary action, (e.g. blood clotting) pre-treat meant is required.
Luminescent immune assay	Chemiluminescence or fluorescencelabelled antibody or antigen	Highly sensitive, Quantitative	1-2 hrs.	Closed analytical systems required for detection
Microarray	rapid high-throughput detection of viral RNA using cDNA	Highly sensitive, longer target sequences can be detected	1 hr.	Fabrication of cDNA micro array is labor intensive.
CT scan	Computerized tomography of Chest images	Enhance sensitivity of detection if findings combined with RT-PCR results	1 hr.	Indistinguishability from other viral pneumonia other chest complications
Biochemical tests	Blood counts, Lymphopenia, elevated serum enzyme levels, high	Could support routine symptomatic treatment regimens of COVID 19	1-24 hrs	Not conclusive evidence for COVID 19 infections

available for COVID-19 is given in table 1. The urgent need for accurate and rapid diagnosis of SARS-CoV-2 infection remains critical as global healthcare systems continue to operate during the course of the COVID-19 pandemic. In particular, serological and immunological testing of infected asymptomatic and symptomatic individuals, and their close contacts, is expected to be in high demand.

CONCLUSION

The COVID-19 pandemic, caused by the SARS-CoV-2, has resulted in over 17 million confirmed cases and over 7 lakh deaths worldwide in less than six months. The living and working conditions of billions of people worldwide have been significantly disrupted due to different forms of social distancing and lockdowns in many cities. The widespread availability of accurate and rapid testing procedures is extremely valuable in unravelling the complex dynamics involved in SARS-CoV-2 infection and immunity. One of the many challenges in containing the spread of COVID-19 is the inability to identify asymptomatic cases that result in the virus spreading to close contacts. Thus, the global outbreak of COVID-19 has emphasized the importance of the laboratory diagnosis of human coronavirus infections in order to limit the spread as well as appropriately treat those patients who have serious complications. WHO has published a uniform case definition for surveillance and testing for COVID-19 infections. However, the focus on implementing the most reliable diagnostic tools varies in different places. Since COVID-19 is a new nosological entity, there are no data as of yet that would enable the determination of standards for the interpretation of specific diagnostic tests. As with any other infectious disease, the accuracy of each method depends on the method of collecting the material, the quality of the sample, and the equipment applied. Although RT-PCR testing plays a crucial role in accurately detecting SARS-CoV-2 on a case-by-case basis, it also has inherent problems that limit its utility. Current obstacles to the widespread use of RT-PCR testing include a shortage of testing kits and an extended processing period of several hours before results are obtained. Moreover, the results of real-time RT-PCR tests must be cautiously interpreted. A combination of real-time RT-PCR and clinical features, especially CT images, could facilitate better disease management. Proper sampling procedures, good laboratory practice standards, and using high-quality extraction and a real-time RT-PCR kit could improve the approach and reduce inaccurate results. Loop-Mediated Isothermal Amplification (LAMP) is a relatively new, convenient molecular amplification point-of-care technique that is widely used for COVID-19 diagnosis. The technique does require sophisticated laboratory equipment to provide similar analytical sensitivity to RT-PCR. Serological testing for COVID-19 is particularly attractive because of the relatively short time to diagnosis and the ability to test for an active

immune response against the virus. A lot remains to be understood regarding the value of serological testing in COVID-19 diagnosis and monitoring. The results of serological tests can then be used to estimate the true spread of the virus through a population, even if individuals were asymptomatic or were never diagnosed. More comprehensive evaluations of the performance of serology tests are rapidly under way. Considerations for the use of serology methods for COVID-19 require the correct and appropriate interpretation of the results and understanding the strengths and limitations of such tests.

REFERENCE

- Abdalahamid, B., Bilder, C. R., McCutchen, *et al.*, (2020). Assessment of Specimen Pooling to Conserve SARS-CoV-2 Testing Resources. *American Journal of Clinical Pathology*, 153(6),715–718. <https://doi.org/10.1093/ajcp/aqaa064>
- Broughton, J. P., Deng, X., Yu, G., *et al.*, (2020). CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, 38(7), 870–874. <https://doi.org/10.1038/s41587-020-0513-4>
- Carter, L. J., Garner, L. V., Smoot, J. W., Li, Y., *et al.*, (2020) Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Central Science*, 6(5),591–605. <https://doi.org/10.1021/acscentsci.0c00501>
- CDC Centre of Disease control and prevention (2020): Coronavirus disease 2019 (COVID-19). Testing for COVID -19
- Centre of Disease Control and Prevention,(2020). Coronavirus disease (COVID-19) guidelines for clinical specimen.,.
- Chan, C. M., Tse, H., Wong, S. S. Y., *et al.*, (2009). Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudo typed virus. *Journal of clinical virology*, 45(1), 54-60.
- Chan, K. H., Poon, L. L., Cheng, V. C. C., *et al.*, (2004). Detection of SARS coronavirus in patients with suspected SARS. *Emerging infectious diseases*, 10(2), 294.
- Chan, M. S., Chan, I. Y., Fung, K. H., Poon, E., *et al.*, (2004). High-resolution CT findings in patients with severe acute respiratory syndrome: a pattern-based approach. *American Journal of Roentgenology*, 182(1), 49-56.
- Chapin, S. C., & Doyle, P. S. (2011). Ultrasensitive multiplexed microRNA quantification on encoded gel microparticles using rolling circle amplification. *Analytical chemistry*, 83(18), 7179-7185.
- Chen, N., Zhou, M., Dong, X., Qu, J., *et al.*, (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, 395(10223), 507-513.
- Cheng, P. K., Wong, D. A., Tong, L. K., *et al.*, (2004). Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *The Lancet*, 363(9422), 1699-1700.
- Cho, C. H., Lee, C. K., Nam, M. H., Yoon, S. Y., *et al.*,

- (2014). Evaluation of the AdvanSure™ real-time RT-PCR compared with culture and Seeplex RV15 for simultaneous detection of respiratory viruses. *Diagnostic microbiology and infectious disease*, 79(1), 14-18.
- Chu, D. K., Pan, Y., Cheng, S. M., Hui, K. P., *et al.*, (2020). Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clinical chemistry*, 66(4), 549-555.
- Corman, V. M., Landt, O., Kaiser, M., *et al.*, (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro surveillance*, 25(3), 2000045.
- Cui, J., Li, F., & Shi, Z. L. (2019). Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, 17(3), 181-192.
- Ding, Y., He, L. I., Zhang, Q., Huang, Z., Che, X., Hou, J., *et al.*, (2004). Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV-2) in SARS patients: implications for pathogenesis and virus transmission pathways. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, 203(2), 622-630.
- Dorfman, R. (1943). The detection of defective members of large populations. *The Annals of mathematical statistics*, 14(4), 436-440.
- Drosten, C., Günther, S., Preiser, W., *et al.*, (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New England journal of medicine*, 348(20), 1967-1976.
- Druce, J., Garcia, K., Tran, T., *et al.*, (2012). Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. *Journal of clinical microbiology*, 50(3), 1064-1065.
- Fouchier, R. A. M., Kuiken, T., Schutten, M., *et al.*, (2003, May). Koch's postulates fulfilled for SARS virus. *Nature*, 423(6937), 240-240. <https://doi.org/10.1038/423240a>
- Francois, P., Tangomo, M., Hibbs, J., Bonetti, E. J., *et al.*, (2011, June). Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. *FEMS Immunology & Medical Microbiology*, 62(1), 41-48. <https://doi.org/10.1111/j.1574-695x.2011.00785.x>
- Gollier, C., & Gossner, O. (2020). Group testing against Covid-19 (No. 24). EconPol Policy Brief.
- Holshue, M. L., DeBolt, C., *et al.*, (2020). First case of 2019 novel coronavirus in the United States. *New England Journal Of Medicine*, 382, 929-936.
- Hong, K. H., Lee, S. W., Kim, T. S., *et al.*, (2020). Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. *Annals of laboratory medicine*, 40(5), 351-360.
- Hsueh, P. R., Hsiao, C. H., Yeh, S. H., *et al.*, (2003). Microbiologic characteristics, serologic responses, and clinical manifestations in severe acute respiratory syndrome. *Emerging infectious diseases*, 9(9), 1163.
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., *et al.*, (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 395(10223), 497-506.
- Kacian, D. L., & Fultz, T. J. (1999). U.S. Patent No. 5,888, 779. Washington, DC: U.S. Patent and Trademark Office.
- Kannan, S. P. A. S., Ali, P. S. S., Sheeza, A., *et al.*, (2020). COVID-19 (Novel Coronavirus 2019)-recent trends. *Eur Rev Med Pharmacol Sci*, 24(4), 2006-2011.
- Kumar, R., Nagpal, S., Kaushik, S., *et al.*, (2020). COVID-19 diagnostic approaches: different roads to the same destination. *Virus disease*, 31(2), 97-105.
- Kwon, K. T., Ko, J. H., Shin, H., *et al.*, (2020). Drive-through screening center for COVID-19: a safe and efficient screening system against massive community outbreak. *Journal of Korean medical science*, 35(11).
- Lamb, L. E., Bartolone, S. N., Ward, E., *et al.*, (2020). Rapid detection of novel coronavirus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PloS one*, 15(6), e0234682.
- Lan, L., Xu, D., Ye, G., Xia, C., Wang, S., *et al.*, (2020). Positive RT-PCR test results in patients recovered from COVID-19. *Journal of American Medical Association*, 323(15), 1502-1503.
- Landreneau, K. J., & Creek, W. (2009). Sampling strategies. Retrieved on: <http://www.natco1.org>
- Lee, J. (2020). COVID-19 screening center: how to balance between the speed and safety? *Journal of Korean Medical Science*, 35(15).
- Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., *et al.*, (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *New England journal of medicine*.
- Li, Z., Yi, Y., Luo, X., Xiong, N., *et al.*, (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of medical virology*, 92(9), 1518-1524.
- Liu, L., Liu, W., Zheng, Y., Jiang, X., Kou, G., *et al.*, (2020). A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. *Microbes and infection*, 22(4-5), 206-211.
- Loeffelholz, M. J., & Tang, Y. W. (2020). Laboratory diagnosis of emerging human coronavirus infections—the state of the art. *Emerging microbes & infections*, 9(1), 747-756.
- Long, C., Xu, H., Shen, Q., Zhang, X., Fan, B., *et al.*, (2020). Diagnosis of the Coronavirus disease (COVID-19): rRT-PCR or CT? *European journal of radiology*, 126, 108961.
- Malik, Y. S., Sircar, S., Bhat, S., *et al.*, (2020). Emerging novel coronavirus (2019-nCoV)—current scenario, evolutionary perspective based on genome analysis and recent developments. *Veterinary quarterly*, 40(1), 68-76.
- Mathuria, J. P., Yadav, R., and Rajakumar .(2020). Laboratory diagnosis of SARS-CoV-2 A review of current methods. *Journal of Infection and Public Health*, 13(7), 901-905.
- Miller, S. E., (2016). Electron Microscopy of Viral

- Infections. Lennette's Laboratory Diagnosis of Viral Infections: Keith R Jerome (ed.) CRC Press.
- Mizumoto, K., Kagaya, K., Zarebski, A., *et al.*, (2020). Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Eurosurveillance*, 25(10), 2000180.
- Monchatre-Leroy, E., Boué, F., Boucher, J. M., *et al.*, (2017). Identification of alpha and beta coronavirus in wildlife species in France: bats, rodents, rabbits, and hedgehogs. *Viruses*, 9(12), 364.
- Nguyen, T., Zoëga Andreassen, S., Wolff, A., & Duong Bang, D. (2018). From lab on a chip to point of care devices: The role of open-source microcontrollers. *Micromachines*, 9(8), 403. 9(8):403.
- Noh, J. Y., Yoon, S. W., Kim, D. J., (2017). Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR. *Archives of virology*, 162(6), 1617-1623.
- Notomi, T., Okayama, H., Masubuchi, H., *et al.*, (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research*, 28(12), e63-e63.
- Nuccetelli, M., Pieri, M., Grelli, S., *et al.*, (2020). SARS-CoV-2 infection serology: a useful tool to overcome lockdown? *Cell Death Discovery*, 6(1), 1-9.
- Okba, N. M., Müller, M. A., Li, W., *et al.*, (2020). Severe acute respiratory syndrome coronavirus 2- specific antibody responses in coronavirus disease patients. *Emerging infectious diseases*, 26(7), 1478-1488.
- Pan, Y., Long, L., Zhang, D., Yuan, T., *et al.*, (2020). Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads. *Clinical chemistry*, 66(6), 794-801.
- Peiris, J. S. M., Lai, S. T., Poon, L. L. M., *et al.*, (2003). Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet*, 361(9366), 1319-1325.
- Pung, R., Chiew, C. J., Young, B. E., Chin, S., *et al.*, (2020). Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures. *The Lancet*, 395(10229), 1039-1046.
- Sethuraman, N., Jeremiah, S. S., *et al.*, (2020). Interpreting diagnostic tests for SARS-CoV-2. *Journal of American Medical Association*, 323(22), 2249-2251.
- Shani-Narkiss, H., Gilday, O. D., *et al.*, (2020). Efficient and practical sample pooling for high-throughput PCR diagnosis of COVID-19. *MedRxiv*.
- Shen, M., Zhou, Y., Ye, J., *et al.*, (2020). Recent advances and perspectives of nucleic acid detection for coronavirus. *Journal of pharmaceutical analysis*, 10(2), 97-101.
- Shi, X., Gong, E., Gao, D., *et al.*, (2005). Severe acute respiratory syndrome associated coronavirus is detected in intestinal tissues of fatal cases. Official journal of the American College of Gastroenterology |. *American Journal of Gastroenterology*. 100(1), 169-176.
- Smyrlaki, I., Ekman, M., Lentini, A., *et al.*, (2020). Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nature communications*, 11(1), 1-12.
- Thai, H. T. C., Le, M. Q., *et al.*, (2004). Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. *Journal of Clinical microbiology*, 42(5), 1956-1961.
- To, K. K. W., Tsang, O. T. Y., *et al.*, (2020). Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *The Lancet infectious diseases*, 20(5), 565-574.
- To, K. K. W., Yip, C. C. Y., *et al.*, (2019). Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. *Clinical Microbiology and Infection*, 25(3), 372-378.
- Tsang, J., & Bajpai, J. (2020). Novel coronavirus infection-knowns and unknowns with particular reference to oncology-combating against covid-19 with "COVID". *Indian Journal of Medical and Pediatric Oncology*, 41(02), 116-120.
- VanGuilder, H. D., Vrana, K. E., *et al.*, (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*, 44(5), 619-626.
- Venter, M., & Richter, K. (2020). Towards effective diagnostic assays for COVID-19: a review. *Journal of clinical pathology*, 73(7), 370-377.
- Verdun, C. M., Fuchs, T., Harar, P., *et al.*, (2021). Group testing for SARS-CoV-2 allows for up to 10- fold efficiency increase across realistic scenarios and testing strategies. *Frontiers in Public Health*, 1205.
- Wan, Z., Zhang, Y. N., He, Z., (2016). A melting curve-based multiplex RT-qPCR assay for simultaneous detection of four human *et al.*, coronaviruses. *International journal of molecular sciences*, 17(11), 1880.
- Wang, D., Hu, B., Hu, C., Zhu, F., *et al.*, (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China: *Journal of American Medical Association*, 323(11), 1061-1069.
- Wang, W. K., Fang, C. T., Chen, H. L., *et al.*, (2005). Detection of severe acute respiratory syndrome coronavirus RNA in plasma during the course of infection.: *Journal of clinical microbiology*, 43(2), 962-965. Wang, W., Xu, Y., Gao, R., *et al.*, (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *Journal of American Medical Association*, 323(18), 1843-1844.
- Wong, M. L., & Medrano, J. F. (2005). Real-time PCR for mRNA quantitation. *Biotechniques*, 39(1), 75-85.
- World Health Organization. (2020). Global surveillance for COVID-19 caused by human infection with COVID-19 virus: interim guidance, 20 March 2020 (No. WHO/2019-nCoV/Surveillance Guidance/2020.6). World Health Organization.
- World Health Organization. (2020). Laboratory testing

- for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020 (No. WHO/COVID-19/laboratory/2020.4). World Health Organization.
- World Health Organization. (2020). Operational considerations for COVID-19 surveillance using GISRS: interim guidance, 26 March 2020 (No. WHO/2019-nCoV/Leveraging GISRS/2020.1). World Health Organization.2020.
- Wu, F., Zhao, S., Yu, B., Chen, Y. M., *et al.*, (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265-269.
- Xiang, J., Yan, M., Li, H., Liu, T., *et al.*, (2020). Evaluation of enzyme-linked immunoassay and colloidal gold-immunochromatographic assay kit for detection of novel coronavirus (SARS-Cov-2) causing an outbreak of pneumonia (COVID-19). *MedRxiv*. <https://doi.org/10.1101/2020.02.27.20028787>
- Xu K, Cai H, Shen Y, *et al.*, (2020). Management of Coronavirus Disease 2019 (COVID-19): Infectious Microbes & Diseases. <https://doi.org/10.1097/IM9.0000000000000023>.
- Xu, M., Ye, J., Yang, D., *et al.*, (2019). Ultrasensitive detection of miRNA via one-step rolling circle-quantitative PCR (RC-qPCR). *Analytica Chimica acta*, 1077, 208-215.
- Yu, F., Yan, L., Wang, N., Yang, S., *et al.*, (2020). Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. *Clinical Infectious Diseases*, 71(15), 793-798
- Yu, H., Sun, B., Fang, Z., *et al.*, (2020). Distinct features of SARS-CoV-2specific IgA response in COVID-19 patients. *European Respiratory Journal*. <https://doi.org/10.1183/13993003.01526-2020>
- Zhang, F., Abudayyeh, O. O., & Gootenberg, J. S. (2020). A protocol for detection of COVID-19 using CRISPR diagnostics. A protocol for detection of COVID-19 using CRISPR diagnostics, 8.
- Zhang, Y., Chen, C., Zhu, S., *et al.*, (2020). Isolation of 2019-nCoV from a Stool Specimen of a Laboratory-Confirmed Case of the Coronavirus Disease 2019 (COVID-19). *China CDC Weekly*, 2(8), 123-124. <https://doi.org/10.46234/ccdcw2020.033>
- Zhong, N, S., Zheng, B. J., *et al.*, (2003). Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, *The Lancet*, 362(9393), 1353-1358. [https://doi.org/10.1016/s0140-6736\(03\)14630-2](https://doi.org/10.1016/s0140-6736(03)14630-2)
- Zhou, P., Yang, X. L., Shi, Z. L., *et al.*, (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579, 270-273. <https://doi.org/10.1038/s41586-020-2012-7>