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REVIEW ARTICLE

A short review on COVID-19 and its diagnosis method

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus has spread to almost every country in the world, which causes fever, headache, dry cough, muscle, and chest pain. The disease potentially affects the heart, renal, and liver processes along with respiratory failure. SARS-CoV-2 viruses cause major problems to global health services. During the pandemic, diagnosis of SARS-CoV-2 infection was often confused with seasonal Influenza viral infection. However, a latent number of methods have been developed for a specific diagnostic method of SARS-CoV-2 infection. This review article compiled the different developed diagnostic methods for easy, rapid, and cost-effective identification of diseases. This article may serve as a ready reference to the researchers working in the field of SARS-CoV-2 infection.

KEY WORDS: COVID-19, Diagnostic method, Reverse transcription polymerase chain reaction, Severe acute respiratory syndrome-coronavirus-2, Serology test, Virus detection

INTRODUCTION

The first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) incident happened at initial December 2019 throughout Wuhan, Hubei Province, when many patients and healthcare workers were found infected with the pneumonia-like viral infection. The World health organization (WHO) announced the incident as a "Public Health Emergency of International Concern." The WHO formally announced the emergence and named the disease SARS-CoV-2 on January 30, 2020. SARS-CoV-2 is 96% identical to a bat-derived SARS-like coronaviral disease and shares 79.6% of its structural similarities with SARS-CoV.[1,2]

Fever is the primary and most ordinary condition of SARS-CoV-2 along with others such as dry sneezing, exhaustion, difficulty breathing, decreased appetite, pressure in the chest, and high temperature. Numerous different clinical signs may impact several other patients but that are less popular including, flavor damage, conjunctivitis (also known as red eyes), nasopharyngeal congestion, throat discomfort, migraine, and aches in the muscles or joints, there are various kinds of skin rashes, nausea, or diarrhea. Direct contact with infected people or by airdrops is the most common method of individual transmission. The greatest chance of transmission is within one meter of an infectious individual; moreover, the maximum range is still unknown.

Coronaviruses are enclosed RNA viruses with a diameter of 60–140 nanometers with a crown-like appearance. It is well known that coronaviruses exhibit mutant and recombined behavior, as an outcome of which lung, digestive, kidney, liver, and neurologic chronic conditions develop. There are seven main coronavirus types identified, including HKU1, NL63, 229E, OC43, severe acute respiratory, MERS-CoV, and SARS-CoV-19.[3,4]

The SARS-CoV-2 epidemic required immediate hospital treatment of the patient, which helps in preventing the disease transmission to others. Reliable, early, and precise diagnosis is also essential for further spreading the infections. Due to the dangerous effect of SARS-CoV-2, the advancement of effective, quick, accessible, and accurate

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diagnostic testing for SARS-CoV-2 disease is urgently needed. Numerous diagnostic assays for immunological and nucleic acid amplification have been established and are easily accessible. Several integrated devices are being developed to provide reliable and rapid clinical services for SARS-CoV-2 diseases. The SARS-CoV-2 infections test such as molecular analysis (nucleic acid amplification), reverse transcription polymerase chain reaction (RT-PCR) test, serological test, CT scan test, chest X-ray, and antigen test are important for the treatment of SARS-CoV-2 virus. This review article highlights various diagnostic methods of SARS-CoV-2 infections being developed for easy, fast, and precise identification. Special emphasis was given to the mechanism involved in the particular diagnosis method for easy understanding.

STRUCTURAL PROTEINS OF SARS-COV-2

SARS-CoV-2 has been confirmed as just a β – coronavirus by analysis of its sequence of nucleic acids. The positive strands of the huge SARS-causing CoV-2 virus contain 29891 nucleotides and 9860 amino acids. This genome is enclosed inside proteins with circular nucleocapsids, which are then enveloped. The SARS-CoV-2 includes different types of proteins such as spike protein (S), nucleocapsid protein (N), envelope protein (E), and membrane protein (M). These proteins served a variety of functions.

Spike protein

Envelopes of SARS-CoV-2 have surface glycoproteins which are called spike proteins, which can recognize the host's binding site, adhere to it, and help the microorganisms enter in the presenter cell wall.[5] A coronavirus name was considered after the crown-shaped structure of the S protein.[6]

Nucleocapsid protein

The N protein, one of the most prevalent viral proteins, is expressed in the presenter specimen. This is understood that it binds to viral RNA and forms a ribonucleoprotein's core, which also aids in virus entry and conversation to cellular functions after virus internalization with the host cell.[7]

Envelope protein

The E protein is a tiny membrane protein that contains an N-terminal domain, a non-polar domain, a loop at the C terminus, and a molecular weight of 8e12 kDa.[8] Moreover, targeting the E protein may result in more effective treatments for SARS-CoV-2.

Membrane protein

Of all proteins found in coronavirus, the M protein is the most prevalent. It possesses a length of around 220–260 amino acids, with such a narrow N terminal domain connected to domains with three transmembrane domains. Furthermore, they are also attached to a carboxyl-terminal domain, making them N-linked glycosylated proteins with a 12 amino acid residue conserved domain.[9]

SARS-COV-2 DIAGNOSIS

Diagnosis is necessary for combating the SARS-CoV-2 virus. Identification and treatment of infected persons, monitoring, randomized controlled trials, and decisions regarding public health are all examples of public health judgment all depend on quick and precise diagnostic testing. Laboratory testing may be done to screen asymptomatic patients or to respond to clinical presentations. Here, we are discussing the several diagnosis methods being used in SARS-CoV-2 infectious diseases.

Clinical diagnosis

The clinical diagnostic tests for SARS-CoV-2 include a basic assessment of possible SARS-CoV-2-related side effects**.** These need to be taken into account within the contextual condition of the SARS-CoV-2 incubation period, which is anticipated to last up to 14 days after exposure, with a median of 4–5 days.[10,11] The American centers for disease control list these 11 common SARS-CoV-2 symptoms; fever, difficulty breathing, exhaustion, muscle pain, headaches, a net loss of smell or taste, a throat infection, congestion or a runny nose, a throat infection, motion sickness or vomiting, and diarrhea.[12] Illness and sneezing are among the major side effects, according to hospital admission information.[10] These symptoms may be used for the identification of the SARS-CoV-2 infection.

In vitro diagnostics molecular testing

Molecular analysis (nucleic acid amplification)

The WHO and Food and Drug Administration's proposed various methods for SARS-CoV-2 identification.[13,14] The nucleic acid amplification tests (NAATs) are single-molecule analysis that examines specimens from patient populations with a high degree of suspicion for a certain viral or bacterial nucleic acid chain. The NAAT of SARS-CoV-2 isolates particular viral genes from the suspected patient's respiratory tract, including the nucleocapsid (N) genotype, the enclosure (E) genotype, the S genotype, and the RNA-dependent RNA polymerase. Thereafter, the molecular biology technique known as true reverse transcription reaction is used to enhance the bacterial infection RNA (RT-PCR). For the purpose of identifying SARS-CoV-2 in patients' respiratory systems, numerous scientists, and producers of medical devices have created a variety of SARS-CoV-2 molecular diagnostic kits.

RT-PCR

RT-PCR is a method of gene transcription that consists of several stages, each with its own set of ambient situations. Overall, RT-PCR is the process of converting RNA into the plasmid sequence (cDNA), accompanied by sample processing and amplification. To obtain scientific evidence of SARS-CoV-2 performance by amplifying particular portions of the plasmid cDNA using a fluorophore-quencher technique.[15,16]

The very first step is to extract ribonucleic acid from the upper or lower respiratory tracts. Upper respiratory specimens such as nasopharyngeal swab samples, oropharyngeal swabs, as well as nasopharyngeal bleaches have been advised. Moreover, through coughing sick people, the sample is normally taken from the lower respiratory tract, which also involves mucus, bronchoalveolar lavage fluid, as well as tracheal swallowing reflex.[17,18] The mixture is put in a thermal cycler and then a sequence of temperature levels and points in time are programmed to function the cycles in RT-PCT. This same fluorophore-quencher detector cleavage produces a fluorescent signal and which even the thermal cycler detects within every cycle, actually providing data regarding the process. Initially, research laboratories checking the virus utilizing the PCR would also deliver outcomes in a short period. The following is a brief overview of applicable RT-PCR detection kits that are currently on the market, along with their corresponding outcome product delivery.

On March 13, 2020, Viractor Erofins introduced the SARS-CoV-2 RT-PCR test. Their RT-PCR test is primarily performed on respiratory system samples such as oropharynx swab samples, nasopharyngeal swabs, nasopharyngeal wash, nose swab, and nose clean; however, it can also be performed on lower respiratory samples such as a bronchoalveolar lavage swab. Suspicion patients are instructed on better sample categories for test results.[19]

On April 24, 2020, BGI Genomics Co. Ltd. (Shenzhen, China) obtained permission for the Real-time Fluorescent RT-PCR Kit for SARS-CoV-2 determination. This same kit contains an automatic vehicle sample collection mechanism, a ribonucleic acid extraction kit, and a PCR framework that can produce consistent outcomes for 192 samples in about 4 h. Top and lower respiratory specimens can be collected using oropharynx swab samples, nasopharyngeal swabs, nose washes, nose sputum, and bronchoalveolar lavage fluid. This kit's cross-reactivity with more than 50 pathogenic organisms was reviewed, and no cross-reactivity was discovered among the pathogens tested.[20]

Bosch developed a 95% accurate, completely automated quick test for SARS-CoV-2 identification that satisfies WHO performance standards. Their instrument is made up of two components: a cartridge involving all or most of the necessary reactants and the Vivalytic analysis software. One such device, which appears as the first fully computerized SARS-CoV-2 test, can be used immediately by health centers to identify the viral infection and provide the results in digital format in <2 h and 15 min. According to reports, the machine can examine a single sample for SARS-CoV-2 as well as nine some pathogenic organisms like influenza A and B.[21]

RT-LAMP

In some molecular-based advanced technology, nucleic acid amplification is conducted isothermally and with much less complex parameters, with the exception of PCR-based techniques, and it needs a thermal cycler since they involve many stages that operate at various temperatures. RT-LAMP has been shown to be an effective technique for broad-scale testing of the viral infection and is a quick and accurate way for identifying SARS-CoV-2. Low background signal, simple accessibility, highly accurate identification than PCR-based methods, and lack of a thermal cycler are all advantages of using RT-LAMP for SARS-CoV-2 testing.[22] To amplify the sequences, the DNA polymerase is utilized with several primers, such as six or four inside as well as outside primers.[23] Reverse transcription is performed in the RT-LAMP first stage to create a DNA structure, as well as the LAMP method is then used in the second stage to amplify the target DNA. RNA is reverse-transcribed into cDNA in the first phase. A DNA structure resembling a dumbbell is produced in the second step of nucleic acid amplification, and this is dependent on automatic cycling strand displacement inner as well as outer primers work together to synthesize DNA. The dumbbell-shaped with numerous sites for DNA synthesis start is converted during amplification into long concatemers; each of which has additional sites. This final step results in the development of many DNA structures with the matching target DNA sequence which can be identified in real-time based on turbidity or after amplification using agarose gel testing.[24] The results of RT-LAMP are assessed by the change in color, fluorescence, or turbidity in the PCR tubes, making it an easy as well as an effective method.[25]

SHERLOCK

Along with the procedures already mentioned, it is another approach that can be utilized to detect the SARS-CoV-2 virus. Clustered regularly spaced short palindromic repeats and nucleic acid amplification are both essential components of the SHERLOCK test. Enzymology is used by the CRISPR-associated system (Cas) to accurately identify the specific target nucleic acid. The guide RNA and Cas nuclease are combined in CRISPR-Cas systems. The CRISPR RNA, which is complementary to the target RNA, and the trace RNA, which functions as a scaffold for Cas nuclease, are the two main parts of the guide RNA, which detects the specific target sequence. CRISPR-Cas technology enables the focused and incredibly sensitive

detection of nucleic acids due to its programmable endonuclease ability.[26] Depending on the significance of outcome delivery speed and sensibility, SHERLOCK can be carried out in a one- or two-step reaction. The one-step reaction may produce results with a femtomolar to the attomolar range of sensitivity in 15–30 min, whereas the two-step reaction can achieve this with a zeptomolar range of sensitivity in 30–60 min.[27]

Immunoassays

It is also used to detect the existence of the SARS-CoV-2 antigen or antibody created to fight against SARS-CoV-2. Numerous researchers and companies that produce medical devices have developed and assessed COVID-19 immunoassays to determine the existence of pertinent antigens or antibodies in COVID-19 sufferers.

Serology (antibody) test for SARS-CoV-2

Serologically, also known as antibody tests, diagnose immune globulins generated by the presenter's blood plasma B cells in response to exposure to an allergen. The SARS-CoV-2 genome contains around 25 protein molecules needed for infectious disease as well as reproducibility, in addition to four significant structural proteins: spike (S), nucleocapsid (N), envelope (E), and membrane (M). The S protein is necessary for fusing and entering the presenter cell, and it contains an S1 receptor-binding domain at its N-terminus, as well as S2 substituents with such an N-terminal domain and a C-terminal site. This is the main component of SARS-CoV-2 N.[28,29]

According to a study conducted on recoverable SARS-CoV-2 patients' serum, Host-neutralizing antibodies target both S and N protein molecules as their primary targets.[30,31] As a result, trying to predict immune defense conditions in serological tests targeting specific regions of S or N protein molecules may become more feasible. Because of this, identifying certain SARS-CoV-2 antigen domains targeted by the humoral immune system has become a crucial part of the development of serological testing. Rapid diagnostic tests (RDT), enzyme-linked immunosorbent assays (ELISA), chemiluminescence immunoassays (CLIA), and neutralization assays are the 4 kinds of serological diagnostic tests. The immobilization screening test is a lab-based procedure used to examine whether a person's antibodies are potentially capable of defending against viral disease *in vitro*. It uses real-time virus and tissue culture procedures. To culture SARS-CoV-2-infected cells, this test, which takes 3–5 days, must be conducted in laboratories with designated biosafety documents. RDTs are quick and simple tests using only a small sample of blood. RDT screening strips determine the presence of patient monoclonal antibodies (IgG, IgM, or IgA) generated against a particular SARS-CoV-2 allergen. An RDT is easy to use and produces results in between ten and thirty min. As a result, a wide range of serological surveys may be conducted. The ELISA assay, a lab-based test that typically takes 2–5 h to complete, is presently the most widely used kind of serological test. In most cases, an ELISA uses an outer layer covered with a particular viral antigen(s) to connect and identify patient antibodies (IgG, IgM, or IgA) in blood, plasma, or tissue samples. The connected antigen-antibody is complicated and then is detected using a 2nd antibody and a substrate on which it produces a colon- or fluorescent-based signal. ELISA tests are classified into three types: Direct, competent, and sandwich or double-antigen-bridging assay processes. CLIA technology is similar to ELISA in that it relies on the strong congeniality between the viral antigen(s) and presenter monoclonal antibody to generate a positive notification, but it employs chemical tests that emit light as a result of a chemical reaction. CLIA is completed in about 1–2 h. These CLIA and ELISA tests all have a high standard of analytical concurrence and are performed in laboratories.[32,33]

Antigen test for SARS-CoV-2

A SARS-CoV-2 antigen test detects coronavirus proteins inside the mouth and the throat. That whole experiment was conducted to investigate whether such a patient is presently contaminated with SARS-CoV-2. The antigen test method is an appropriate "first line of defense" test against SARS-CoV-2, especially in individual people with SARS-CoV-2 signs, due to its relatively simple procedure that is faster than that of other methods, such as PCR analysis; an outcome is usually generated within about fifteen min. A specimen of a patient's throat and nasal passages is commonly used in SARS-CoV-2 antigen test cases (the sample can also be saliva or blood). The specimen is again analyzed in the same way that a test result. Outcomes do seem to be probably accessible within 15–30 min after the specimen is evaluated, so a patient on-site may very well have their outcomes before they leave.[34]

Other methods for SARS-CoV-2 diagnosis

The SARS-CoV-2 diagnosis is done by other different types of methods, and these methods played a significant contribution and cleared the path for rapid and accurate COVID-19 detection. These are the following methods:

Computed tomography (CT) scan test for SARS-CoV-2

CT scan has been used as an adjunct strategic plan for early clinical diagnosis and assessment, and X-rays may support symptoms suggestive of SARS-CoV-2. Radioactivity assessments, especially narrow segment chest CT scans, are critical during the diagnosis, regulation, and surveillance of SARS-CoV-2 infectious diseases.[35] In situations where the imaging irregularity is diagnosed early, pneumonia may be suspected. Even though SARS-CoV-2 identification is ultimately based on RT-PCR, tools and techniques are

essential for pneumonia diagnosis. CT scans are suggested if there are suspicious lung abnormalities. Correct diagnosis of SARS-CoV-2 pneumonia may outcome in motivating treatment and follow-up. Radiologists should be aware of radiological reports because lung scanning exposes the severity of SARS-CoV-2. Clinical results from SARS-CoV-2 patients brought to the intensive care unit (ICU) with developing anomalies on CT scans.[36]

On hospitalization, such patients had subsegmental centralized management and diplomatic numerous papillary, as a result, non-ICU patients received subsegmental centralized management as well as diplomatic ground-glass opacity (GGO).[37] Scanning can confirm non-homogenous centralization with GGOs in bi-lateral lungs as well as bronchiectasis in serious infections, indicating "white lung." whereas most lung lobes are directly.[38]

People with the disease SARS-CoV-2 may also suffer from intralobular septal stiffening, bilateral pleura, and even a small quantity of pleural effusion.[39] CT needs to allow for early diagnosis of respiratory problems and quick patient safety response. Slice chest CT has been found to be the main argument for approved observational data. Hence, even though chest X-rays are insensitive for the order to diagnose GGO and may start producing clear results in the initial stages of the disease, it isn't recommended.[40] This is not the primary imaging technique for SARS-CoV-2. All the same, in serious conditions, bi-lateral varifocal integration may be seen, mainly fused into the larger centralized control with mild pleural effusions and occasionally displaying "white lung."[39] In this particular with regard to the thin-slice chest CT measure is significantly much beneficial in identifying SARS-CoV-2 bacterial infection.[35]

High-resolution CT could indeed diagnose GGOs.[4] A large-scale study (The disease has been confirmed in 3665 people) discovered that 95.5% have pneumonia. Pan *et al*. carried out a study with 21 authorized SARS-CoV-2 situations who underwent repeated CT at approximately 4-day durations and identified negative outcomes in 4 cases in the early stage (0–4 days from the beginnings of the initial side effects) but replicated CT demonstrated irregularities in the respiratory system in all sick people did admit.[41]

Moreover, because SARS-CoV-2 CT results overlap with those from other influenza viruses' cases of pneumonia, the RT-PCR test is highly recommended for quick treatment and detection.[42]

SARS-CoV-2 chest X-ray

X-ray computer systems are commonly available all over the world as well as it produces an image quickly, a few more medical researchers suggested chest scans. Due to its effectiveness, affordability, and low price, an X-ray is normally the very first imaging technique in patient populations with people accused or proven SARS-CoV-2, even if it has a lower response than a CT scan.[43]

Conducting X-rays in ordinary apartments places non-infected sick people as well as diagnostic imaging assistants in danger due to the possibility of transmission of infection through the droplet-contaminated outer surface.[44] As a result, this same apartment should be decontaminated after every use.[44,45]

Creating a specialized ordinary diagnostic imaging room for everyone SARS-CoV-2 patient can help reduce transmission, but not all centers have the resources to do so.[46] Using a multifunction system to perform an anteroposterior (AP) estimation of the X-ray helps to prevent the spread of infectious disease. Since this can be easily handled and located in identified facilities for SARS-CoV-2 sick people. It thus minimizes the necessity to move possibly infectious sick people throughout the health center, including the use of personal safety equipment.[44,43]

This is also the only one that is capable of being conducted on critically ill as well as ICU sick people. Its analysis is frequently strictly controlled either by a smaller degree of imagination as well as the magnifying of something like the cardio-mediastinal silhouette generated by that of the AP projection. However, besides its restrictions, it allows for the assessment of catheter and instrument position, the identification of complications associated (such as pneumothorax, subcutaneous emphysema, and pneumomediastinum), and the sequence surveillance of human disease progression.

To aid in the diagnosis, chest X-ray observations in sick persons with evidence of SARS-CoV-2 have indeed been divided into four types.

- An ordinary chest X-ray – A normal chest X-ray does not really control infectious diseases because it is not particularly unusual for the X-ray to really be an ordinary initial in the illness.[43,47]
- A reticular sequence, GGOs, and restructuring, as well as a shaped morphological characteristic and a lobulated or fragmented varifocal distribution, are examples. The spread is typically two-way and peripheral, with both the lower fields predominating.[43] Organizing pneumonia, drug interactions, and some other causes of severe respiratory problems are all possibilities in the diagnostic process. During the 1st and 3rd weeks after symptoms start, results from traditional X-ray studies may indicate diffuse disease. This is a serious clinical hypoxemia situation, with the severe syndrome of respiratory distress as the major differential diagnosis (ARDS).
- Intermediate observations, which also appear in situations of SARS-CoV-2 pneumonia, may be generated by other factors. Restructurings and GGOs

with such a conditional, middle, or upper-lobe spread are examples of these infectious diseases, and alveolar edema is on the list of possible diagnoses.

- A typical, unusual, or unreported research results in SARS-CoV-2 pneumonia. This included lobar centralization, respiratory system nodules or masses, current military sequence, delamination, and pleural effusion, which have been confirmed in only 3% of patients 23 and are much more common in progressive disease.

CONCLUSION

This paper discusses how India is preparing to deal with a growing number of SARS-CoV-2 case scenarios, actually dealing with the current scenario, including such negative impacts on the economy, human living, and also the environment during the SARS-CoV-2 lockdown period, and alternative methods taken to combat this pandemic. This paper also gives the knowledge of the identification of many other virus infections, particularly SARS-CoV-2, It has significantly contributed to the development of the quick and accurate SARS-CoV-2 diagnostic test. The latest techniques and approaches for SARS-CoV-2 diagnosis have been discussed here like the RTPCR test, serological test, CT scan, antigen test chest X-ray, and many more in terms of their fundamentals of operating conditions. Because detection methods are really an important aspect of dealing with pandemics, it is necessary to tackle the difficulties of current approaches, grow extra efficient ways, and diagnose all infected people quickly and accurately in the early phases of SARS-CoV-2.

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