Diverse Techniques Applied for Effective Diagnosis of COVID-19

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1 Introduction

Coronaviruses are single-stranded RNA viruses with genome size ranging from 26 to32 KB [7]. They have spikes that project from their surfaces. In humans, they cause respiratory tract infection which can be mild or severe. Mild infection could show symptoms such as common cold, while the severe form could cause severe acute respiratory syndrome (SARS), Middle East Respiratory Syndrome (MERS), and COVID-19 infection [52]. SARS-CoV-1 was the first strain of the SARS coronavirus identified to be responsible for the outbreak that occurred between the years 2002 and 2004 [9, 49]. In December 2019, another strain of SARS coronavirus

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broke out in the City of Wuhan in China. This strain was named SAR-CoV-2 which causes Coronavirus disease 2019 (COVID-19) [22–24].

Some of the diagnostic techniques that could be applied for quick identification of SARS-CoV-2 include reverse transcription polymerase chain (RT-PCR), real-time quantitative polymerase chain reaction (RT-qPCR) [22], an enzyme-linked immunosorbent assay (ELISA), IITF, lateral flow immunoassay, and clustered regularly interspaced short palindromic repeats (CRISPRs) [50]. In addition, imaging techniques such as the computerized tomography (CT) and X-rays could also be utilized to diagnose the disease [23, 24]. All the methods highlighted above could be affected by inadequate sample volume, inappropriate collection of samples, inaccuracy of the technique, inappropriate window for the sample collection, and contamination [26, 36]. There is a need for the development a rapid and cheaper, way to diagnose the disease. Furthermore, there is need to develop home-based kits that can be used by individuals to know their status and immediately self-isolate if confirmed positive. Even though vaccines are now available that can prevent infestation of this dangerous virus, the role of diagnosis of the disease in prevention of the spread of the virus cannot be overemphasized. Moreover, the application of molecular and advanced biotechnological techniques has been documented to play a crucial role for rapid detection of COVID 19 diseases [1, 4, 20].

Therefore, this chapter intends to provide a detailed information on the application on some techniques that could be applied for adequate and quick detection of COVID 19 diseases.

2 General Overview on COVID-19

The term “severe acute respiratory syndrome coronavirus 2” used in depicting the virus clearly indicates the lungs are the major hubs affected by the virus. For COVID-19, symptoms appear between 1 and 14 days following exposure to the virus. It is worth noting that 33% of people infected usually do not present with noticeable features. The virus spreads through contact, infected surfaces,

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aerosols, and respiratory droplets. Even though, symptoms appear variable, most of the widely reported symptoms include pneumonia, shortness of breath, cough, anosmia, sore throat, and many other symptoms [38]. Pathophysiologic mechanism involves modulation of renin-angiotensin-aldosterone system [35]. Specifically, the virus invades the host cells through angiotensin converting enzyme II, an enzyme that is present in large quantity in pneumocytes [51]. Hence therapeutic goals antagonizing angiotensin II activity might prove helpful [15]. As expected, innate immunity, specifically inflammatory response occurs during viral invasion. However, in COVID-19, there appears to be an association between COVID-19 severity and the levels of inflammatory cytokines such as monocyte chemoattractant protein-1, gamma-interferon, interleukin-IB, and many more. This implies that the barrage of diseases characterizing the condition is attributable to severe inflammatory responses [43].

3 COVID-19 and Mental Health

A Chinese study showed that 96.2% of COVID-19 inpatients experienced post-traumatic stress symptoms [6]. Even in healthcare providers, a study reported that the prevalence of anxiety and depression stands at 45% and 51%, respectively [22–24]. A study conducted in Ibadan, Nigeria, on the health implication of COVID-19-induced lockdown on adult residents revealed that sleeplessness correlated positively with anxiety and depression [2]. In Spain, an elevated level of anxiety and stress was orchestrated by COVID-19 lockdown measures in young children [46]. About one quarter of older people with depression may be adversely affected by COVID-19 measures, most especially, social distancing measures.

4 COVID-19 Diagnosis and Management

Identification of COVID-19 has been done using real-time polymerase chain reaction (TaqPath COVID-19 kit), transcription-mediated amplification, reverse transcription loop-mediated isothermal amplification, and nasopharyngeal swab [3]. Serology test which detects serum or plasma antibodies is also available [45]. Non-invasive techniques such as chest computerized tomography, computerized tomography, and X-rays are also beneficial as far as examination of the pulmonary structure is concerned.

One of the ways to prevent COVID-19 is through social distancing (at least 6 feet). Other preventive strategies include regular hand washing, avoiding unnecessary movement, social gathering restrictions, and use of face mask. Face mask was found very effective as a preventive measure [17]. In addition, vaccines are now available to induce an artificial active immunity against the virus [5]. However, it has been observed that many COVID-19 patients have benefited from numerous anti-

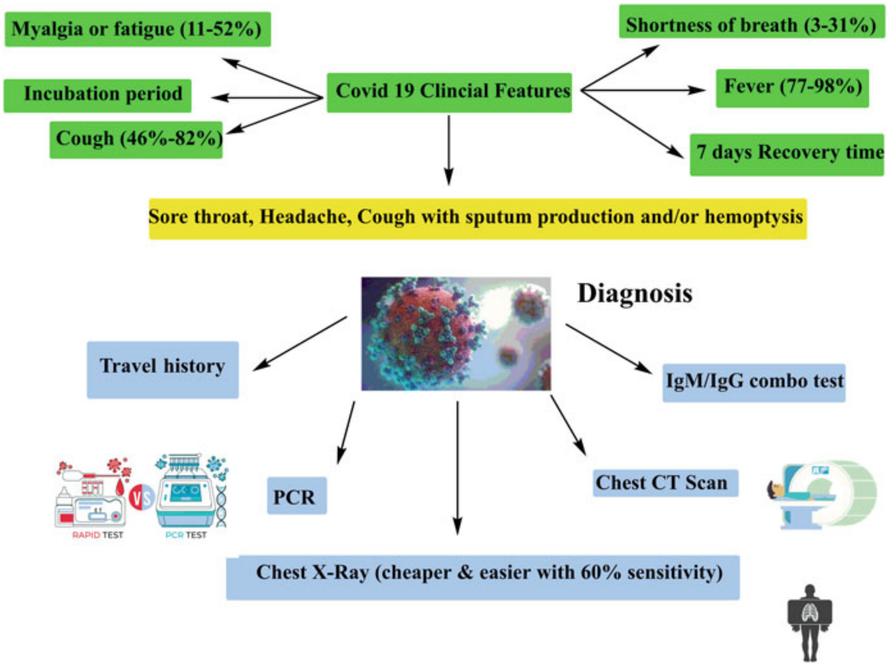

Fig. 1 Several techniques that could be applied for the identification of COVID-19

COVID-19 drugs such as vasodilators, antiviral drugs, corticosteroids, and many more. Figure 1 illustrates diverse COVID-19 clinical features, symptoms, and the various types of techniques applied for the detection of COVID-19.

5 Different Comprehensive Techniques for Rapid Detection of COVID-19

Prompt diagnosis of COVID-19 is vital to prevent the spread of the disease. Diagnosis of COVID-19 involves using different techniques to identify the occurrence of the virus using various samples. Antibodies play a momentous function in the detection of infectious pathogens. Once there is an exposure to SARS-CoV-2, the body's immune system produces antibodies (human IgG) which can be detected between the 7th and 14th days after infection and may even persist after the clearance of the virus [25]. Enzyme-linked immunosorbent assay (ELISA) detects the presence of human IgG against SARS-CoV-2 in the serum or plasma. As described in [33], patient samples and standards are placed in microplate wells containing specific bound antigens in the well surface. An enzyme conjugate is added and allowed to incubate for 60 mins at room temperature in order to form antigen-antibody complex. The mixture is washed 4-5 times using wash buffer to remove unbound materials. A Tetramethylbenzidine (TMB) substrate is added and the mixture is incubated for another 20 mins; this causes changes in color of the mixture. The intensity of the color corresponds to the quantity of the human IgG in the sample. A stop is added and the absorbance read at 450 nm wavelength using the microplate reader. The concentration of the antibodies can be obtained by using absorbance of the sample and the standard. The real-time reverse transcription polymerase chain (rRT-PCR) can be used for qualitative detection of SARS-CoV-2 nucleic acid [41]. Samples from oropharyngeal and nasopharyngeal swabs, sputum, and aspirate from lower respiratory tract are used. The RNA from the virus is made undergo reverse transcription to cDNA which is amplified using a thermal cycler. During amplification, probes bound to template are cut off using Taq enzyme. The polymerase chain reaction (PCR) instrument will draw a real-time amplifier curve based on the signal received, and this will either show the occurrence or absence of novel SARS-CoV-2. The CRISPR-Cas systems allow for easy diagnosis of COVID-19 in less than 40 mins. This method has advantage over rRT-PCR because of its ability to simultaneously perform reverse transcription and thermocycling amplification using loop-mediated replication for SARS-CoV-2 RNA [53]. The Cas systems detect the viral sequences when the presence of the virus is confirmed. The system is rapid, sensitive, and the reporting format is user's friendly. Some of the other diagnostic techniques that has been used to check for the presence of SARS-CoV-2 in samples include indirect immunofluorescence assay, lateral flow assay, X-rays, and the use of computerized tomography.

6 Performance of Several Laboratory Diagnostic Evaluations and Platforms

Diagnosis of COVID-19 involves confirmation of coronavirus using molecular technique. Also test that detects serum antibodies against the virus (serological tests) is also available. With respect to molecular test, the challenge is the time required for the result to be available. For serological tests, the issue is the sensitivity of the technique. Yet rapidity and sensitivity of diagnosis are crucial in the prevention of disease progression and community transmission.

As part of measures to unravel the challenge, Biotechnology Companies are working very hard to ensure rapid techniques are developed. An example of this is a new multiplex real-time polymerase chain reaction developed by Thermo Fisher Scientific called TaqPath COVID-19 kit. The kit contains both controls and assays that are required for the detection of ribonucleic acid from coronavirus-2019 virus.

Many studies on COVID-19 diagnosis identified RT-PCR as a consensus technique for COVID-19 validation. Sensitivity of this technique is dependent on specimen type. For instance, analysis of sputum specimens yielded a sensitivity of 69%, while that of nasopharyngeal swabs yielded 63% [22–24]. The time the specimen is collected is very crucial. Samples collected earlier before disease onset or later may yield a false negative result. Evidence exists to prove that sensitivity of the test using sputum specimen is dependent on the timing of sample collection. Moreover, it has been demonstrated that COVID-19 normally shows the greatest viral load during the first week after the onset of symptom and decline thereafter [22–24].

7 Alternative Methods for the SARS-CoV-2 Detection

The application of molecular analytical test has become highly relevant in the identification of COVID-19 disease. The development of these tests is based on proper understanding of protein and genetic make-up of SARs-CoV-2 that causes infection. RNA is an ideal biomolecule that promotes the nucleic acid specificity via base pair complementarity, as reported by RNA interference or modification study [31]. These properties of RNA and other similar biomolecules have transformed molecular diagnosis by promoting a quick and precise detection of nucleic acid for infectious pathogens such as SARs-CoV-2. Among the relevant molecular techniques used in COVID-19 identification are CRISPR-based high sensitivity enzymatic test, RNA aptamer, DETECTR, and next-generation gene sequencing.

8 CRISPR-Based Techniques

A special member of the CRISPR-associated (Cas) proteins, Cas13, can be used to identify the viral molecules of COVID-19 under the influence of a predesigned guide CRISPR RNA (crRNA), which in turn initiates trans-cleavage of reporter-coupled single-strand nucleic acids by Cas13 to produce fluorescent signals or readable colorimetric signals on a lateral flow strip. Using this method, crRNA sequences are specifically selected to reduce relevant sequence related to the genomes of other human respiratory viruses [8, 32]. Thus, this test can constantly detect SARS-CoV-2 target sequences and present the test outcome in few minutes [8, 54, 55].

CRISPR-associated (Cas) is a gene editing technology. In the diagnosis of COVID-19, many CRISP-based methods are available as potential options. All-in-One Dual CRISPR Cas 12a, unarguably, is a very accurate and highly sensitive technique. It has been designed for identification of COVID-19 virus [19]. One of the advantages of AIOD-CRISPR could applied in point-of-care screening [12]. CRISP Cas 12-based detection developed by Broughton and colleagues is very rapid and takes less than 40 minutes compared to other isothermal nucleic acid-oriented techniques. It has the potential of being used to diagnose COVID-19 from RNA samples of COVID-19 patients [10].

9 DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR)

Another technique that can be utilized for the diagnosis of COVID-19 is called DNA endonuclease targeted CRISPR trans reporter (DETECTR) assay [8] with accuracy level comparable to real-time polymerase chain reaction with an advantage of thermocycling avoidance. Besides this, it is also less complex, easy, and portable.

10 CAS 13-Based Rugged Equitable Scalable Testing (CREST)

A very sensitive, easy, and portable method called Cas 13-based Rugged equitable scalable testing (CREST) was introduced by Rauch et al. [44]. It is a cheap technique that makes use of available protein.

10.1 Amplification-Free Assay

Amplification-free assay is a mobile phone-oriented diagnostic method which detects COVID-19 virus in patient's nasal swab [16]. It is a portable and affordable technique.

10.2 Specific High Enzymatic Reporter Unlocking

This is a new diagnostic method based on real-time reverse transcriptase developed by Jasper Fuk-woo and has been shown to be more specific and sensitive than RdRp-p2 approach. This is a lateral flow-based assay which was developed by Zheng's laboratory with promising roles in timing and cost economy and highly sensitive pathogen detection [32]. It has been discovered that with the aid of CRISPR, it is possible to detect COVID-19. This is because the approach is more sensitive with a higher level of specificity when compared to real-time polymerase chain reaction using metagenomics [16].

10.3 Post Analysis Phase

It has been discovered that reporting, verification, interpretation, and documentation of COVID-19 results take place during the post analysis phase. This phase is as important as the previous two phases.

10.4 RNA Aptamers

Aptamers comprise of nuclei acid oligonucleotides or small peptide molecules with high specificity for binding specific target molecules, thereby resulting to their increased sensitivity and precise detection. As a result of their high reproducibility and purity as well as increase stability and reversibility, with high presence of target molecules, aptamers are considered as a new diagnostic device [37]. For adequate diagnosis of COVID-19, aptamers are used together with RT-PCR, or ELISA and are used as aptamer-linked immune sorbent assay, and cantilever-based aptasensors. The increased sensitivity of aptasensors as well as quick and easy diagnostic process is highly important in point-of-care diagnosis of SARs-CoV-2. This alternative test has been applied to diagnose Norovirus strains and bovine viral diarrhea efficiently relative to PCR test [14, 39]. With COVID-19 infestation, aptamers that recognize the viral protein has been used quick identification of SARs-CoV-2 and may act as an antiviral agent in treating COVID-19 disease [11, 57].

10.5 Next-Generation Sequencing (NGS)

NGS techniques such as (Explify®) are sophisticated molecular procedures that started in 2005 and currently used in genomic research. Till date, this technique has become a gold standard in genomic sequencing and very useful in the detection of several diseases and genetic mutations (Morozova and Marra [30]. NGS DNA sequencing is majorly applied to provide adequate information on the molecular prevalence, spread, and classification of pathogens. Compared to single gene analysis done by other tests, a single NGS test can analyze large volume of gene present in the clinical sample, thus, making this technique to be highly resourceful and generally accepted as a diagnostic tool, transforming the diagnosis of pathogens. NGS technology together with other bioinformatics tools has revolutionized the study of viral parthenogenesis and diagnostics and has useful application in the current SARs-CoV-2 outbreak [28]. Thus, NGS technique has a great potential to identify unknown mutation or DNA recombination in the gene of SAR-CoV-2 within a little period of time, thereby improving the diagnosis and preventing a second or third wave as well as new outbreak of infection.

11 Molecular Diagnostic Techniques for COVID-19

COVID-19 molecular diagnosis is done using real-time polymerase chain reaction, reverse transcription loop-mediated isothermal amplification, and transcription-mediated amplification using nasopharyngeal swab. COVID-19 diagnosis consists of preliminary phase, sample analysis phase, and post-analysis phase.

11.1 Preliminary Phase

The preliminary phase consists collection of sample and sample transportation and storage. Collection of samples requires trained personnel under strict observance of standard precautionary and preventive measures. It is imperative to take note that the higher the amount of viral RNA, the higher the likelihood of COVID-19 detection. Samples required for molecular COVID-19 diagnosis are nasopharyngeal and oropharyngeal swabs, sputum, bronchial lavage, saliva, blood, and many more [56]. In Nigeria, the National Center for Disease Control stipulates that one oropharyngeal swab and one nasopharyngeal swab be collected for the test.

11.2 Analysis Phase

There are several laboratory methodologies that are available for diagnosis. The major concern of molecular diagnosis is the timing of test result. The knowledge that there exist some COVID-19 patients who practically display no symptoms of the disease (asymptomatic COVID-19 patient) altered the perception mode regarding diagnosis and highlighted the need for more effective diagnostic tools. This will help in not only early and prompt diagnosis of COVID-19 in symptomatic patients but also prediction of disease progression and prognosis. Early and prompt detection of COVID-19 is critical in order to recognize the unsuspecting population who are at risks of developing disease symptoms and early institution of treatments. As a highly contagious disease, prompt diagnosis requires alteration in traditional orientation regarding laboratory setting which mandates that tests should be done only in specific laboratory locations. Having COVID-19 screening in point-of-care or bed sites demands molecular techniques suiting mobile diagnostic laboratory model will help enormously. There are many methods including immune assays which work by detecting DNA or RNA in the sample make COVID-19 diagnosis faster.

11.3 Loop-Mediated Isothermal Amplification (LMIA)

LMIA centers on selective amplification at a constant temperature of specific nucleic acid. With the method, denaturation of initial template is not important. Hence this method offers a prompt, sensitive, and less expensive diagnosis. The procedure is economical because it does not involve the use of costly reagents and instruments. There exists modified loop-mediated isothermal amplification. One of them integrates reverse transcription with conventional loop-mediated isothermal amplification [22–24]. The procedure based on reverse transcriptase-LMIA has been showed to be relatively consistent with 88.89% sensitive when compared to real-time polymerase chain reaction technique without consuming much time [18]. LMIA works based on the principle of colorimetry which has advantage of minimizing cross contamination [27].

Numerous scientists have validated the significant LMIA and other molecular techniques in the identification and diagnosis of COVID-19 [13, 21, 29, 34, 40, 42, 47, 48].

12 Conclusion and Future Perspectives

This chapter has given a comprehensive detail on diverse facts on approaches that could be utilized for quick recognition and diagnosis of COVID-19. Relevant information was also provided on COVID-19 and mental health, COVID-19 identification and handling, and different comprehensive techniques for SARS-CoV-2 detection. Moreover, it was also established in this chapter that the application of molecular approaches has been identified as a sustainable diagnostic test that could perform a momentous function in the identification of COVID-19 disease. Typical examples of such molecular techniques highlighted in this chapter includes CRISPR-based high sensitivity enzymatic test, RNA aptamer, DETECTR, and next-generation gene sequencing. There is a need to increase the awareness about the application of next-generation sequencing most especially in the developing countries for swift identification of COVID-19 diseases.

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