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Review article

A scoping review of SARS-CoV-2 diagnosis: current options and future aspects

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ABSTRACT

Background: SARS-CoV-2 which causes COVID-19 can infect people with moderate to severe illnesses. In the months following the pandemic's initial detection in China in December 2019, it has quickly spread over the globe. The virus has continued to be prevalent in numerous countries with different mutated strains associated with variable degrees of clinical symptoms despite significant attempts to limit the disease. A coordinated strategy incorporating precise epidemiology, surveillance, prophylaxis, and surveillance is necessary to limit this pandemic. Therefore, accurate diagnosis utilizing quick technology is essential. The precise and timely diagnosis of SARS-CoV-2 is crucial for the efficient care and prevention of COVID-19 patients as well as to stop the transmission of the disease in light of the rising incidence of COVID-19 cases. The various diagnostic techniques for the diagnosis of COVID-19 infection in both clinical and research settings are described in the current review. This article specifically describes the technical and instrumental aspects of the diagnostic techniques employed. The diagnostic methods are all covered in updated and comprehensive detail.

Introduction

Early diagnosis and isolation of suspected patients with COVID-19 play a vital role in controlling this outbreak [1]. The specificity and sensitivity of various diagnostic procedures varies depending on the population and type of equipment used [2].

Epidemiological data, clinical symptoms, and some adjuvant technologies, such as nucleic acid detection and immunological tests, are utilized to make a COVID-19 diagnosis. In addition, high-throughput equipment (biosafety level-3) is required for the isolation of SARS-CoV-2 to ensure worker safety [3].

Despite significant attempts to limit the illness, the virus has remained widespread, so quick and proper diagnosis is crucial. Individuals with new SARS-CoV-2 (or COVID19) have a wide range of symptoms, ranging from asymptomatic to acute respiratory distress syndrome and multi-organ failure [4]. As a result, diagnosing COVID-19 accurately is difficult. COVID-19 is routinely diagnosed based on epidemiological history, clinical signs, and laboratory detection methods such as

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nucleic acid amplification test (NAAT), and serological approaches [5].

Specimens such as nasopharyngeal and/or oropharyngeal swabs, bronchoalveolar lavage fluid, sputum, bronchial aspirate, or blood are typically advised for early screening or diagnosis of SARS-CoV-2 infection [6]. For an accurate and timely identification of the causal agent, laboratory testing, in addition to clinical and epidemiological studies, is critical. This is also known to help with quarantine effectiveness [7].

The virus is spreading locally, but there is just a modest innate immune response. Nasal swabs can identify the virus at this stage. These people are infectious, even if their viral burden is low. The viral RNA RT-PCR result may be beneficial in predicting viral load, future infectivity, and clinical course. These investigations may be able to detect super spreaders. The sample collecting process would have to be standardized. Swabs from the nose may be more sensitive than swabs from the throat [8].

Overall, the scientific community has developed various approaches beneficial for appropriately detecting a suspected case of COVID-19 infection in a short period of time. However, not only the test to be utilized, but also the patient's medical history, the timing of the suspected SARS-CoV-2 exposure, the type of sample to be obtained and analyzed, and how to interpret the result are all factors to consider when diagnosing COVID-19. Only by combining all of these factors will it be feasible to make an accurate diagnosis and successfully control the COVID-19 pandemic [9, 10].

The technical and practical components of the applied diagnostic approaches are specifically described in this article. All of the diagnostic techniques are addressed in depth and with the most recent information.

Clinical presentation

The majority of people infected with the virus will get a typical cold or flu such as fever, cough, myalgia, sore throat, anosmia, fatigue, headache, and chills; with only a few remaining asymptomatic. The condition will manifest itself in minor symptoms in 80% of patients [11]. A recent study of nearly 140 patients at Wuhan University's Zhongnan Hospital identified a variety of symptoms that led to the development of COVID-19. Nearly all of the patients developed a fever with an extremely high temperature, while more than half of the patients also experienced fatigue and a dry cough. A dry cough and trouble breathing affected one-third of the patients [12].

According to the Chinese CDC, around 80% of coronavirus infections are light, 15% of patients have developed severe cases, and 5% of patients have been dangerously sick. A day-by-day analysis of coronavirus symptoms demonstrates how symptoms increase in average people and how COVID-19, the disease, progresses from poor to worse [13].

Day 1: The patient has fever, weariness, muscular discomfort, and a dry cough on the first day of the symptom. A few of them may have nausea and diarrhoea a few days before symptoms emerge. Day 5: Patients may experience breathing difficulties, particularly if they are old or have a medical condition. Day 7: These are the symptoms that led to the patient being admitted to the hospital, according to a Wuhan University research. Day 8: According to the Chinese CDC, 15% of patients get acute respiratory distress syndrome (ARDS), a disease in which fluid builds up in the lungs and is usually deadly. This is more common in severe situations. Day 10: As the illness progresses, the symptoms increase, and the patient is transferred to the intensive care unit (ICU). Patients with milder symptoms are more likely to experience stomach discomfort and hunger loss. The current mortality rate is around 2%. Day 17: Patients who recover are usually discharged from the hospital after two and a half weeks [11].

However, identifying symptoms in the early stages of an infection can be challenging [14]. For verified coronavirus disease 2019 cases, symptoms have varied from mild to severe sickness and death. COVID-19 emergency warning indications include constant discomfort or pressure in the chest, difficulty breathing, disorientation, and pale lips or face, all of which require rapid medical intervention. Pneumonia develops when the disease worsens [15]. Symptoms might develop as soon as three days after exposure or as late as 13 days later. According to new study, the incubation time is roughly five days on average [12].

Serological diagnosis

One of the most significant diagnostic tools in disease monitoring is detecting Viral-specific antibodies in infected patients. Though RT-qPCR is the most widely used method for diagnosing SARS-CoV-2 active patients, viral RNA becomes nearly

undetectable 14 days after infection [16]; moreover, false-negative findings might occur owing to inappropriate viral sample handling. These difficulties necessitate the development of simple test kits that identify human antibodies produced in response to viral infection. The detection of antibodies generated in response to viral infection (IgG and IgM) and/or viral antigen using enzyme-linked immunosorbent assays is the basic premise underpinning antibody-based immunodiagnostic (ELISA).

Antigen-specific antibodies can be discovered in a patient after 3 to 6 days, while IgG can be detected later in an infection, according to studies [16]. These assays may be used to offer information on both present and previous illnesses, and they can be scaled up to evaluate thousands of samples in labs with limited resources. It may also be used in disease monitoring programs to have a better knowledge of the infection rate in the community. Although serological assays may detect both ongoing and previous infections, their effectiveness in verifying SARS-CoV-2-specific antibody responses to detect past infections is well established [16,17].

A study in China found that the titer of virus-specific antibodies in asymptomatic COVID-19 patients is much lower than in symptomatic COVID-19 patients [18]. The average time it took to identify IgM and IgA antibodies in symptomatic COVID-19 patients was 5 days, and 14 days for IgG. After 5.5 days of symptom onset, IgM ELISA had a greater detection efficiency than RT-qPCR [19]. The presence of IgM antibodies implies current viral infection, whereas the presence of IgG antibodies indicates past SARS-CoV-2 infection. As a result, immunodiagnostic tests are also crucial for the development of COVID-19 vaccines [7].

There have been reports of a wide variety of virus-neutralizing antibodies, and new research shows that they may correspond with the severity of disease but fade with time [20,22].

Quick antigenic and rapid antibody tests are faster than RT-PCR-based procedures, with execution durations of 15-30 minutes, a cheaper cost, and a simpler approach [23].

The low viral load and low antibody response observed in some patients are primarily responsible for the low sensitivity and high false-negative results; however, as previously stated the likelihood of obtaining a positive test is also dependent on the time of the presumed infection and the test execution time. Indeed, while viral antigens can be identified in samples within a short period of time following infection, their longevity and stability in biological samples are restricted, making it difficult to appropriately identify these proteins [24].

Rapid antibody tests, meanwhile, are primarily intended to detect IgM and IgG antibodies, which are not created by the body right away but begin to appear in the bloodstream during the third week of a suspected infection. As a result, depending on the period of the suspected infection, it's critical to employ the most appropriate test. Recently, it has been recommended to employ quick testing for the identification of IgA to speed up the diagnosis of COVID-19 infection [25,26].

Radiological findings

Radiological investigations help clinicians to correctly diagnose COVID-19 infection in the suspicious case of pneumonia. Chest X-ray (CXR) and computed tomography (CT) are the most powerful radiological imaging tools for diagnosing COVID-19 pneumonia [27]

Chest X-ray

In the early stages of the disease, a chest X-ray is frequently inconclusive and may not reveal any major alterations. Bilateral multifocal alveolar opacities develop as the infection advances, which may be coupled with pleural effusion [28]. CXR is commonly used to diagnose pulmonary abnormalities after lung damage caused by infectious or cancerous disorders [29-30]. CXR was frequently employed to detect multifocal opacities affecting mostly the lung interstitial space and alveoli in patients with COVID-19-related symptomatology during the initial phase of the COVID-19 epidemic [31].

CXR is mostly used for patients with moderate to severe symptomatology who are suspected of COVID-19 infection and have interstitial opacities (71.7%) or alveolar opacities (60.5%), which typically involve both lungs (64.5%) [32]. These radiological abnormalities worsen with time as symptoms worsen, and they are most commonly seen in older individuals with preexisting pulmonary parenchyma changes (such as patients with chronic obstructive pulmonary disease) who have both bilateral interstitial and alveolar opacities [33].

Computed tomography

CT Even in the early stages of the disease, high-resolution CT (HRCT) is the tool of choice for detecting COVID-19 pneumonia. Multifocal bilateral 'ground-glass' regions associated with consolidation and a patchy peripheral distribution, with increased involvement of the lower lobes, are the most typical signs. A reversed halo sign,' defined as a central region of patchy opacities surrounded by a peripheral ring with consolidation, is also present in certain cases. Pleural effusion, cavitation, calcification, and lymphadenopathy are among the other findings [28].

One of the earliest live imaging tools for detecting pneumonia-related infections is chest computed tomography (CT). It has previously been frequently utilized to detect lung anomalies in SARS and MERS, and has been proven to be more sensitive than X-rays [34]. The approach has recently been used in hospitals for the diagnosis of COVID-19. The approach, however, has its own set of constraints. For example, chest radiography had a sensitivity of 69 % in a retrospective study of 64 patients in Hong Kong, compared to 91 % in RT-PCR. On a chest radiograph, 20% of the RT-PCR positive subjects did not reveal any lung abnormalities [35].

In another research, 75 % of RT-PCR negative patients had chest CT abnormalities, with 48 % of them expected to be COVID19 positive (Ai et al., 2020). Furthermore, because it can overlap with other illnesses including influenza, SARS, and MERS, chest computed tomography alone could result in false positive results. In light of these considerations, the majority of health commissioners have lately dropped chest CT scanning as diagnostic criteria for suspected COVID-19 cases. However, employing a combination of chest CT scans and RT-PCR methods, these diagnostic uncertainties can be efficiently resolved. Furthermore, chest CT imaging might be beneficial in clinical settings for monitoring COVID-19 development and therapy impact [7].

Despite the inexpensive cost and quick radiological results achieved by CRX, some lung abnormalities are not clearly apparent by this method. As a result, in addition to CRX, CT scan is commonly used to better visualize lung abnormalities, which are mostly characterized by bilateral interstitial ground-glass opacities. The CT scan, in particular, has a high resolution power and a sensitivity of 95-100 %, but the specificity is limited since this technology does not allow for the differentiation of pulmonary abnormalities associated with various etiological agents other than SARS-CoV-2 [36].

Molecular diagnosis

Since viraemia is typically detected early in the course of an illness, nucleic acid amplification tests (NAAT) are the most sensitive assays and frequently used test to identify early viral infections. Many NAAT techniques, including reverse transcription real-time PCR (RT-qPCR), loop-mediated isothermal amplification-based assay (RT-LAMP), microarray, and high-throughput sequencing, have been developed for the quick and accurate diagnosis of COVID-19. On the other hand, SARS-CoV-2 RNA of the highest caliber is needed for NAAT. As advised by the WHO and CDC, probe-based RT-qPCR has long been considered the gold standard method for identifying SARS-CoV-2 and is currently one of the most widely used assays for population screening in many countries [37-38]

Several RT-qPCR techniques were employed after the initial epidemic to locate SARS-CoV-2 in clinical samples. RT-qPCR experiments were used to target the RNA dependent RNA polymerase (RdRp), nucleocapsid (N), envelope (E), spike (S), and ORF1b or ORF8 regions of the SARS-CoV-2 genome [39]. The WHO recommends employing an RT-qPCR-based assay targeting the E gene for SARS-CoV-2 screening, followed by a confirmatory test targeting the RdRp gene. The CDC advised utilizing an RT-qPCR test that used the N1 and N2 nucleocapsid protein genes [40].

The upper respiratory tract is sampled using nasopharyngeal and oropharyngeal swabs, while the lower respiratory tract is sampled using expectorated sputum and bronchoalveolar lavage (only for mechanically ventilated patients). The samples are delivered to the laboratory after being kept at 4°C for amplification of the viral genetic material via a reverse-transcription procedure. This entails either reversetranscription PCR (RT-PCR) or real-time RT-PCR to create a double-stranded DNA molecule from the existing viral RNA [41].

In situations of a positive test, the test should be repeated for confirmation, as well as to confirm viral clearance in COVID19 positive patients. The sensitivity of these tests is low in the early infection; for example, 53.3 % of COVID-19-

confirmed patients had positive oropharyngeal swabs, and 71 % of COVID-19-confirmed patients had positive RT-PCR results with sputum samples [42]. After 2–8 days, the RT-PCR findings are frequently positive [43].

For a mean of 17 days, reverse transcription polymerase chain reaction (RT-PCR) tests can detect viral SARS-CoV-2 RNA in the upper respiratory tract; however, detection of viral RNA does not always imply infectiousness, and viral culture from PCR positive upper respiratory tract samples has only been positive once beyond nine days of illness [44].

The gold standard approaches for making a confirmed diagnosis of COVID-19 infection are RT-PCR-based molecular assays [45]. Since the complete sequencing of the SARS-CoV-2 genome [46], researchers from various countries have started developing molecular primers and probes specific to SARS-CoV-2 RNA sequences in order to distinguish COVID-19 infections from other pathologies with similar symptoms, such as seasonal flu or bacterial infections [47-50].

Because of the low sensitivity of the primers and probes used, or the inaccuracy of the entire RT-PCR procedure, a significant fraction of COVID-19-positive patients were identified as false-negative during the early stages of the pandemic, when diagnostic techniques had not yet been optimized and standardized (false-negative rates ranging from 38 % at the day of symptom onset to 67 % before one day from the onset of symptoms [51].

Viral culture and electron microscopy

Viral culture has represented the fundamental method that allows the identification of SARS-CoV-2 as a novel causative agent of human pneumonia [52]. Despite the difficulty of obtaining a viral culture in vitro and the length of time required, viral isolates constitute a watershed moment in the identification of new viral infections [53]. In the case of SARS-CoV-2 infection, viral culture was critical in the early stages of the outbreak before alternative diagnostic tests were developed.

Zhu and his colleagues, 2020 were the first to isolate SARS-CoV-2 virus isolates from clinical material and use transmission electron microscopy to investigate cytopathic effects. After this study, additional research groups isolated SARS-CoV-2 with the goal of studying its structural properties and molecular interaction with infected cells [54]. Other cell lines, such as the Vero and LLC-MK2 cell lines, have been used for these purposes; using electron microscopy and cells infected with clinical specimens obtained from COVID-19 patients, it was possible to identify the virus's ultrastructural details, the virus's interaction with cells, and the resulting cytopathic effects [55].

It's worth noting that electron microscopy was one of the first approaches for discovering new diseases, allowing structural traits to be identified. Solid-phase immune electron microscopy (SPIEM) and immunolabeling electron microscopy (IEM), which are based on the observation of cells blocked in the surface of a grid and the observation of antibody-antigen complex occurring in infected cells, respectively, are the two main applications of electron microscopy in viral infections [56].

In general, viral culture and electron microscopy are crucial approaches for observing the virus's main properties. These two approaches were used to identify the usual structure of coronaviruses, which is characterized by a nucleocapsid encased inside a crown-like envelope made up of spike proteins in the case of SARS-CoV-2. In terms of cytopathic consequences, both approaches showed a wide spectrum of cellular changes, the most prominent of which was the creation of plaques with a net-like structure or joined cells. Deformed cilia with a granular structure and disorganized polarity are also observed in these plaques, which are made up of multinucleated syncytial cells. Double-membrane vesicles and damaged mitochondria were also seen in SARS-CoV-2-infected cells. Finally, viral infections caused the endoplasmic reticulum to expand and the number of secretory vesicles to grow [55].

Despite their relevance, both viral culture and electron microscopy have drawbacks that restrict their application in therapeutic settings. Viral culture is time-consuming and needs specialized equipment as well as a high level of biosecurity. As a result, the CDC recommends using SARS-CoV-2 virus culture only in laboratories with level 3 biosafety cabinets for research purposes [57]. Electron microscopy, on the other hand, is not frequently utilized since it needs expensive gear and highly educated workers with particular abilities in sample preparation and image interpretation. Furthermore, this method has a limited diagnostic sensitivity and specificity, and the best findings can

only be achieved if adequate viral cultures are available [46, 57].

Conclusions

There are three main concerns in the diagnosis of COVID-19: reducing the number of false negatives by detecting small amounts of viral RNA by PCR; avoiding the number of false positives by correctly distinguishing positive signals from different pathogens; and capacity for testing a large number of samples quickly and accurately.

Finding antibodies against a virus in infected people is one of the most important diagnostic techniques in disease surveillance. Although RT-qPCR is the most popular technique for identifying SARS-CoV-2 active patients, viral RNA almost disappears 14 days after infection, and improper viral sample processing may result in false-negative results. Due to these challenges, straightforward test kits that can detect human antibodies produced in response to viral infection are urgently needed. The fundamental idea behind antibody-based immunodiagnosis is the use of enzyme-linked immunosorbent assays to identify antibodies (IgG and IgM) produced in response to viral infection and/or viral antigen (ELISA).

RT-PCR which has drawbacks such the requirement for costly equipment, skilled workers, and poor sensitivity and accuracy. Numerous serological, fast antigen and biosensor-based assays have been authorized for the detection of SARS-CoV-2. Technologies for accurate, specific and sensitive detection of SARS-CoV-2 are being developed and include improved nucleic acid based methods like NASBA and RT-LAMP, CRISPR-Cas and its variations, nanobodies based LFA, SPR assays, paper assays, semiconductors based binding assays, use of aptamers functionalized with quantum dots and employing functionalized nanostructures in order to improve the sensitivity of PCR based methods. Diagnosis will be increasingly prevalent in the future, particularly in the event of global pandemics like COVID-19, thanks to the combination of cutting-edge molecular diagnostics, artificial intelligence, and LFAs.

References

1-**Team E.** The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020. China CDC weekly 2020;2:113.

2-**Leeflang MM, Rutjes AW, Reitsma JB, Hooft L, Bossuyt PM.** Variation of a test's sensitivity and specificity with disease prevalence. Cmaj 2013;185:E537-44.

3-**AL-Khikani FH.** COVID-19: Containment strategies and management options. Journal of Nature and Science of Medicine 2020;3:221.

4-**Al-Khikani FH.** Mucormycosis "Black Fungus" new challenge associated with COVID 19. Biomedical and Biotechnology Research Journal (BBRJ) 2021;5:267.

5-**Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al.** Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 2020;25:20-25.

6-**Keski H.** Hematological and inflammatory parameters to predict the prognosis in COVID-19. Indian Journal of Hematology and Blood Transfusion 2021;37:534-42.

7-**Rai P, Kumar BK, Deekshit VK, Karunasagar I, Karunasagar I.** Detection technologies and recent developments in the diagnosis of COVID-19 infection. Applied microbiology and biotechnology 2021;105:441-55.

8-**Al-Khikani FH.** Surveillance 2019 novel coronavirus (COVID-19) spreading: Is a terrifying pandemic outbreak is soon. Biomed Biotechnol Res J 2020;4:81-2.

9-**AL-Khikani FH, Ayit AS.** A scoping review of SARS-CoV-2 and male infertility: Concerns and future prospects. Asian Pacific Journal of Reproduction 2022;11:53.

10-**Almosawey HA, AL-Khikani FH, Hameed RM, Abdullah YJ, Al-Ibraheemi MK, Al-Asadi AA.** Tamoxifen from chemotherapy to antiviral drug: Possible activity against

COVID-19. Biomedical and Biotechnology Research Journal (BBRJ) 2020;4:108.

11-**Hafeez A, Ahmad S, Siddqui SA, Ahmad M, Mishra S.** A review of COVID-19 (Coronavirus Disease-2019) diagnosis, treatments and prevention. Ejmo 2020;4:116-25.

12-**Lee JE, Hwang M, Kim YH, Chung MJ, Sim BH, Chae KJ, et al.** Imaging and clinical features of COVID-19 breakthrough infections: a multicenter study. Radiology 2022;303:682-92.

13-**AL-Khikani FH, Kadim MM.** Secondary unculturable bacteria associated with Sars-Cov-2: More information are required. Medical Journal of Dr. DY Patil University 2022;15:S136-7.

14-**AL-Khikani FH.** Non culturable bacteria associated with COVID-19: More details are demanded. Microbes and Infectious Diseases 2021;2:611-2.

15-**Amanatidou E, Gkiouliava A, Pella E, Serafidi M, Tsilingiris D, Vallianou NG, et al.** Breakthrough infections after COVID-19 vaccination: Insights, perspectives and challenges. Metabolism open 2022;17:100.

16-**Lee HK, Lee BH, Seok SH, Baek MW, Lee HY, Kim DJ, et al.** Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross reactivity with human coronaviruses 229E and OC43. Journal of veterinary science 2010;11:165-7.

17-**AL-Khikani FH.** Impact of IL-35 and presepsin on immunological, hematological, and biochemical parameters in COVID-19 patients. Infection Epidemiology and Microbiology 2023;9:144-150

18-**Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al.** Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature medicine 2020;26:1200-4.

19-**Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al.** Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clinical infectious diseases 2020;71:778-85.

20-**Cevik M, Kuppalli K, Kindrachuk J, Peiris M.** Virology, transmission, and pathogenesis of SARS-CoV-2. bmj 2020;3:371.

21-**Al-Janabi AA, Al-Khikani FH.** Prophylaxis and therapeutic ability of inactivated dermatophytic vaccine against dermatophytosis in the rabbits as an animal model. Turkish Journal of Pharmaceutical Sciences 2021;18:326.

22-**Obayes AK.** Amphotericin B from antifungal to antiviral therapy: promising modern therapeutic branch. Research Results in Pharmacology 2020;6:6.

23-**Pilarowski G, Lebel P, Sunshine S, Liu J, Crawford E, Marquez C, et al.** Performance characteristics of a rapid SARS-CoV-2 antigen detection assay at a public plaza testing site in San Francisco. MedRxiv 2020;4:2020-11.

24-**Jacobs J, Kühne V, Lunguya O, Affolabi D, Hardy L, Vandenberg O.** Implementing COVID-19 (SARS-CoV-2) rapid diagnostic tests in Sub-Saharan Africa: a review. Frontiers in medicine 2020;18:684.

25-**Al-Hussainy AD, AL-Khikani FH, Hussein AZ, Alshamary RS.** Correlation between severe acute respiratory syndrome Coronavirus-2 and cytomegalovirus. Medical Journal of Dr. DY Patil University 2022;15:S286-90.

26-**AL-Khikani FH.** Viruses and male infertility: Where we are now?. Microbes and Infectious Diseases 2022 Nov 19.

27-**Chang MC, Lee W, Hur J, Park D.** Chest computed tomography findings in asymptomatic patients with COVID-19 Respiration. 2020;99:748-54.

28-**Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R.** Features, evaluation, and treatment of coronavirus (COVID-19). Statpearls [internet]. 2022 Feb 5.

29-**AL-Khikani FH.** Pulmonary mycoses treated by topical amphotericin B. Biomedical and Biotechnology Research Journal (BBRJ) 2020;4:123.

30-**Al-Khikani FH.** Amphotericin B as antiviral drug: Possible efficacy against COVID-19. Annals of thoracic medicine 2020;15:118.

31-**Larici AR, Cicchetti G, Marano R, Merlino B, Elia L, Calandriello L, et al.** Multimodality imaging of COVID-19 pneumonia: from diagnosis to follow-up. A comprehensive review. European Journal of Radiology 2020;131:109217.

32-**Ippolito D, Maino C, Pecorelli A, Allegranza P, Cangiotti C, Capodaglio C, et al.** Chest X-ray features of SARS-CoV-2 in the emergency department: a multicenter experience from northern Italian hospitals. Respiratory medicine 2020;170:106.

33-**Lei J, Li J, Li X, Qi X.** CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia. Radiology 2020;295:18

34-**Memish ZA, Al-Tawfiq JA, Assiri A, AlRabiah FA, Al Hajjar S, Albarrak A, et al.** Middle East respiratory syndrome coronavirus disease in children. The Pediatric infectious disease journal 2014;33:904-6.

35-**Wong HY, Lam HY, Fong AH, Leung ST, Chin TW, Lo CS, et al.** Frequency and distribution of chest radiographic findings in patients positive for COVID-19. Radiology 2020;296:E72-8.

36-**Kovács A, Palásti P, Veréb D, Bozsik B, Palkó A, Kincses ZT.** The sensitivity and specificity of chest CT in the diagnosis of COVID-19. European Radiology 2021;31:2819-24.

37-**Chu DK, Pan Y, Cheng SM, Hui KP, Krishnan P, Liu Y,et al.** Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clinical chemistry 2020;66:549-55.

38-**AL-Khikani FH, Alkhafaji ZA.** The rs568408 variant in the IL-12A gene is associated with risk for COVID-19 in Iraqi patients. Tzu Chi Medical Journal 2023 Jan 3.

39-**Reusken CB, Broberg EK, Haagmans B, Meijer A, Corman VM, Papa A, et al.** Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020. Eurosurveillance 2020;25:82.

40-**Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al.** First case of 2019 novel coronavirus in the United States. New England journal of medicine 2020 Jan 31.

41-**Behera B, Rout B, Kar SK, Sahoo D, Sahu KK, Otta S.** ABO blood grouping and COVID-19: a hospital-based study in Eastern India. Egyptian Journal of Medical Human Genetics 2022;23:7.

42-**Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al.** Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerging microbes & infection 2020;9:386-9.

43-**Huang P, Liu T, Huang L, Liu H, Lei M, Xu W, et al.** Use of chest CT in combination with negative RT-PCR assay for the 2019 novel

coronavirus but high clinical suspicion. Radiology 2020;295:22-3.

44-**Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A.** SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The lancet microbe 2021;2:e13-22.

45-**Mahendiratta S, Batra G, Sarma P, Kumar H, Bansal S, Kumar S, et al.** Molecular diagnosis of COVID-19 in different biologic matrix, their diagnostic validity and clinical relevance: A systematic review. Life sciences 2020;258:118207.

46-**Wu SY, Yau HS, Yu MY, Tsang HF, Chan LW, Cho WC, et al.** The diagnostic methods in the COVID-19 pandemic, today and in the future. Expert review of molecular diagnostics 2020;20:985-93.

47-**Al-Khikani FH.** The role of blood group in COVID-19 infection: More information is needed. Journal of Nature and Science of Medicine 2020;3:225.

48-**Al-Khikani F, Ayit A.** The Antibacterial Action of Safranin and Gentian Violet. Rambam Maimonides Medical Journal 2022;13:66

49-**AL-Khikani FH, Ayit AS.** Pseudomonas Aeruginosa a tenacious uropathogen: Increasing challenges and few solutions. Biomedical and Biotechnology Research Journal (BBRJ) 2022;6:311.

50-**AL-Khikani F.** Factors affecting flowering of Pseudomonas aeruginosa in urine. Microbes and Infectious Diseases 2022 ;3:956-7.

51-**Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J.** Variation in false-negative rate of reverse transcriptase polymerase chain reaction–based SARS-CoV-2 tests by time since exposure. Annals of internal medicine 2020;173:262-7.

52-**Zhu N, Wang W, Liu Z, Liang C, Wang W, Ye F, et al.** Morphogenesis and cytopathic effect of SARS-CoV-2 infection in human airway epithelial cells. Nature communications 2020;11:3910.

53-**Al-Khikani FH, Al-Hussainy AD, Hussein AZ, Alshamary RS.** SARS-CoV-2 and Helicobacter pylori and some hematological parameters: A case–control study. Journal of Medical Society. 2022;36:129.

54-**Leung A, Tran K, Audet J, Lavineway S, Bastien N, Krishnan J.** In vitro inactivation of SARS-CoV-2 using gamma radiation. Applied Biosafety 2020;25:157-60.

55-**Zhao J, Zhou H, Huang W, Zhou J, Qiu M, Deng Z, et al.** Cell morphological analysis of SARS-CoV-2 infection by transmission electron microscopy. Journal of thoracic disease 2020;12:4368.

56-**Akilesh S, Nicosia RF, Alpers CE, Tretiakova M, Hsiang TY, Gale Jr M, et al.** Characterizing viral infection by electron microscopy: lessons from the coronavirus disease 2019 pandemic. The American journal of pathology 2021;191:222-7.

57-**Bain W, Lee JS, Watson AM, Stitt-Fischer MS**. Practical guidelines for collection, manipulation and inactivation of SARS-CoV-2 and COVID-19 clinical specimens. Current protocols in cytometry 2020;93:e77.