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Diagnostic Tests for SARS COV-2: Application, Challenges and Recommendations

Mohibullah Salih[1], Delaqa Waqar[2], Hasibullah Baha Nijrabi[3], Oniba Saiqal[2]

[1]Department of Microbiology, Kabul University of Medical Sciences, Kabul, Afghanistan, [2]Department of Islamic Studies, Kabul University of Medical Sciences, Kabul, Afghanistan, [3]Department of Forensic Medicine, Kabul University of Medical Sciences, Kabul, Afghanistan

mohibullahsalih786@gmail.com

Abstract. **Background**: The recent pandemic of human coronavirus infections, at the beginning of the 21st century, have shown the prominent roles of rapid and accurate diagnostic technologies to contain the emerging and re-emerging infections. To control such a large-scale crisis, early identification and prevention of infection are essential. Prompt identification of cases relies on the accurate diagnostic technologies. Before analysis, appropriate sample collection is essential for making a precise molecular diagnosis of SARS-COV-2 (Severe Acute Respiratory Syndrome Coronavirus-2). To prevent further spread of infection and obtain consistent results, proper preventive measures must be taken into consideration to keep laboratory staff safe. Despite the development of specific and sensitive point-of-care tests and serological immunoassays which are considered supplementary methods for diagnosing SARS-COV-2, RT-PCR (Reverse-Transcriptase Polymerase-Chain-Reaction) remains the gold standard performed on respiratory specimens. Upon completion of the sample analysis, testing results must be interpreted using both molecular and serological findings. Finally, random-access, integrated devices available for the purpose of care with scalable capacities will facilitate the rapid and accurate diagnosis and monitoring of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS CoV-2) infections. **Objectives**: This review covers the types of sample collection, current diagnostic techniques for COVID-19 and challenges in their application **Methods**: A literature search was performed on the lines for a narrative review, but including features of systematic review methodology (PRISMA flow diagram). Two authors searched three electronic databases for literature review, i.e., PubMed, HINARI, and Google Scholar using Mesh-terms, specimen types or molecular diagnostic tests or serological tests in link with COVID-19 or SARS CoV-2, with different combinations, between March 21, 2020 and March 8, 2023. **Conclusion**: To control the current pandemic of COVID-19 and reduce its burden on healthcare systems around the world, the capacity of diagnostic tests should be improved. Rapid molecular diagnostic tests that can be fast, reliable, cost-effective, available, sensitive, and specific and point of care, must be urgently developed, produced, and widely distributed all over the globe.

Keywords. COVID-19, SARS CoV-2, Specimen types, Molecular tests, Serological tests, Diagnostic approach, Diagnostic challenges

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1. Introduction

The SARS-CoV-2 virus has emerged in Wuhan, China, and poses a global threat to public health around the world. The most important and appropriate approach to prevent the adverse consequences of viral epidemics requires the development of surveillance programs and necessary laboratory preparations. During a viral pandemic, diagnostic laboratories (using molecular diagnostic methods) play a crucial role in the rapid and accurate identification and isolation of new microorganisms [1]. Besides, the introduction of molecular diagnostic techniques and rapid serological tests can lead to the rapid identification, isolation, and treatment of positive Covid-19 cases[2].

The role of diagnostic tests depends on the type of test available, the resources required to perform the test, and the time required to announce the test result. In other words, the rapid identification of suspected cases is the main goal, to properly observe the use of personal protective equipment (PPE) and to prevent the spread of infection to the hospital and subsequently to the community [3]. The current preferred method for laboratory diagnosis of COVID-19 is the reverse-transcription PCR test [4, 5]. Viral culture is not recommended. On the other hand, some available point-of-care tests (POCT), are based on molecular techniques and are suitable for detecting new cases of Covid-19. While other types of these tests are based on serological methods and are more suitable for determining people who have already been infected [6]. This review discusses the existing diagnostic techniques and Challenges in their implementation.

1.1. Pre-analytic stage

During the first few days of illness (prodromal period), patients with COVID-19 demonstrate high viral load in the upper & lower respiratory tract [7, 8]. For early diagnosing of infection, a nasal/oral swab is recommended [9-11]. The nasopharyngeal swab is preferred because it's safe to the conductor & well tolerated by the patient. A study conducted by Wang et.al. has shown that most of the specimens were collected using OP swabs (n=398) rather than NP swabs (n=8); however, the SARS-CoV-2 RNA was detected in only 32% of OP swabs, which was significantly lower than nasal swabs (63%) [12]. Handling OP/NP swabs carry the risk of transmission of SARS-CoV-2 especially when there is a lack of personal protective equipment (PPE) [13]. In such cases, alternative options; for example, self-collected saliva should be sought [14, 15]. WHO recommends Dacron or polyester flocked swabs for collecting respiratory specimen [16]. After collection, the specimen should be placed in a viral transport medium under the refrigerated condition for rapid transportation to the clinical microbiology laboratories [17]. In some cases, a specimen such as saliva or NP/OP swabs may miss the early infection, and in late stages, the infection may have been shifted to the lower respiratory tract. Hence, repeated tests and sample collection from the lower respiratory tract are recommended in such circumstances. Sputum or bronchoalveolar lavage (BAL) should be used for collecting lower respiratory tract specimens, as a recent study revealed that bronchoalveolar lavage fluid yielded the highest SARS-COV-2 RNA rate although this study did not compare to results from NP swabs[12]. Apart from direct sputum, NP/OP and bronchoalveolar lavage specimens, some patients with COVID-19 demonstrated high RNA loads of SARS-COV-2 in fecal specimen[18, 19]. A recent study carried by Chen, et.al. revealed that 66.6 % of patients (n=28) admitted to the hospital were RNA positive in fecal specimens while 33.3 % (n=14) showed negative RNA results in RT-PCR [20]. Thus apart from direct respiratory sampling, the well-liked method for SARS-CoV-2 in advance COVID-19 cases could also be a rectal swab and real-time RT-

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PCR[21-25]. All respiratory specimens should be processed in BSL-2 [4, 26]. For Extraction of macromolecules, before RT-PCR is performed, the specimen should be transferred to a lysis buffer under a biosafety-level-2 cabinet. This lysis buffer must contain a guanidinium-based inactivating agent and a non-denaturing detergent ready to inactivate any viable coronavirus[27, 28].

There is undeniable evidence that shows that the stage before analysis of experiments is a major source (46 to 68.2% of the total) of errors of medical laboratories [29, 30]. It is estimated that a quarter of all errors before test analysis lead to unnecessary research, inadequate patient care, increased financial burden on the health sector, and ultimately inadequate and slow health care services[31]. Safety and quality of diagnostic tests can be affected greatly by, misdiagnosis of the patient or sample, inappropriate and inadequate preparation of samples, unsuitable conditions for transport and storage of samples (prolonged transfer time or damaged samples), the presence of prohibitive substances in Samples (for example, cellular components due to freezing of blood or unsuitable additives)[32, 33]. and finally, issues related to sample preparation, including errors in pipetting during manual preparation of samples, cross-contamination, and non-compliance of samples[34].

1.2. Analytic stage

1.2.1. Serological diagnostic tests for Covid-19

There are two types of serological tests. Laboratory-based & point-of-care serological assays. Immunoassays are designed to detect either antigen (SARS-COV-2 RNA) or antibodies (IgM, IgG) and are generally lateral flow assays. These rapid antigen lateral flow assays are providing fast results and have low cost, but have poor sensitivity in detecting early infection[35, 36].

Serological and point of care tests are being developed to diagnose positive cases, identify asymptomatic patients and those who are in the convalescence period. Serological tests have several advantages over real-time RT-PCR: Serological tests can provide more details by identifying people who have antiviral-specific antibodies in their blood serum. If these tests are positive, they indicate that the person has passed the infection[37], so they can provide better information about the prevalence of infection in a community [19, 38]. Antiviral-specific antibodies, unlike viral RNA, remain in a person's blood for several weeks to months after the onset of symptoms. When a person's serological results are negative, it means that the person may not have been infected while collecting the sample, but this does not mean that the person will not get sick. Also, the development of antibodies against SARS-CoV-2 does not mean that a person is immune to Covid-19, as many strains of SARS-CoV-2 are not neutralized by antibodies. Given the fact that 20-80% of SARS-CoV-2 positive cases are asymptomatic, in such circumstances the evaluation of the immunity system of individuals in a community by serological tests is valuable. Because serological tests alone are not sufficient to diagnose SARS-CoV-2, the concomitant use of serological tests and molecular diagnostic methods can provide satisfactory results[39].

a. *Manual ELISA*: Different types of ELISA kits are designed to detect neutralizing antibodies (IgM / IgG / IgA) against SARS-CoV-2. Various ELISA kits are also available for coronavirus antigens (SP and NP), but these kits are used for research purposes and have limited values for clinical diagnostic purposes. Despite the current problems, serological tests using the ELISA test still play a major role in the diagnosis and control of the current pandemic. Therefore, the development of ELISA hand kits as a complementary test for real-time RT-PCR

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and the removal of some of its shortcomings and limitations in the future is still a top priority [40].

b. *Automated serology*: The growing demand for diagnostic tests on the population of communities imposes a large clinical and economic burden on diagnostic laboratories. The usage and implementation of serological diagnostic tests have increased the quality assurance and reduced the time for the samples to return, as well as reduced the false-negative and false-positive results. Automated techniques are now common in most serological tests. Conventional serological tests, which are more acceptable than automated tests, are deployed in a laboratory setting to identify individuals' immune status. These tests are very useful when the outbreak reaches its peak. The majority of manual ELISA kits available for SARS-CoV-2 use a 96-well microplate as a solid-phase & standard calorie metric method to receive the signal. While in automated ELISA, solid-phase materials are different, for example, instead of microplates, polystyrene or metal-based nanoparticles (magnetic beads) are used. An automated method, highly sensitive systems such as chemiluminescence technology are used. In April 2020, a fully automated serological test for SARS-COV-2 antibodies was launched. This test was developed to obtain specific IgG antibodies against S1 and S2 domains of spikes proteins of SARS-CoV-2. This increases the specificity of this test and prevents the interaction of different types of coronaviruses and thus avoids false-positive results [41].

c. *Rapid serological test*: Rapid diagnostic tests are designed to evaluate asymptomatic patients who are in the convalescence period. These tests are small and portable and are based on qualitative measurements with either negative or positive results. Some rapid serological tests have used lateral flow techniques. For example, Surescreen Diagnostic COVID-19 IgG / IgM rapid test cassette and Biomedomics rapid IgM-IgG combined antibody test for COVID-19. Others have used time-resolved fluorescence immunoassays. For example, Goldsite Diagnostics Inc. SARS-CoV-2 IgG / IgM kit. All rapid serological tests can detect antibodies from different samples like blood, plasma, or serum. The procedure for all of these tests is the same. for example, taking blood from a patient's finger, adding it to the kit, and then adding a buffer solution to it. The results of these tests take 10-15 minutes [41].

Although analytical errors are the smallest contributors to laboratory errors, several potential analytical problems could significantly jeopardize the quality of testing. These errors include equipment malfunction, non-adequately validated assays, undetected failure of quality control, active viral recombination, testing carried outside the diagnostic window, poor harmonization of primers or probes, and non-specific RT-PCR annealing [32, 33].

1.2.2. Nucleic acid amplification tests (NAAT)

Studies have shown that molecular methods for definitive diagnosis of Covid-19 are more accurate than CT scans and serological tests [42]. Because molecular methods can target and detect SARS-CoV-2 specific antigens. Currently, molecular diagnostic methods (NAAT) for SARS-CoV-2 include real-time RT-PCR (performed in a laboratory setting) and reverse transcription loop-mediated isothermal amplification (RT-LAMP), performed at a patient care facility. Unfortunately, the current diagnostic tests are time-consuming, need professional employees, and human resources, and lastly, lack of adequate diagnostic kits delays the diagnosis [43].

a. *Manual laboratory-based NAAT*: Currently, real-time RT-PCR for various clinical specimens including; broncho-alveolar lavage, lavage fluid, fibrous bronchoscopy biopsy, mucus, nasal swab, throat swab, feces, and blood, is the gold standards for definitive diagnosis of SARS-CoV-2. Real-time RT-PCR has several advantages: It is a specific test that can

differentiate SARS-CoV-2 from similar viruses in the early stages of infection, even when the viral load is low. Thus, unlike serological testing, real-time RT-PCR is of high diagnostic value for virus detection in the early stages of infection[44, 45] (which aims to prevent the spread of infection) because it can directly detect viral RNA even before antibodies are developed in the patient's serum. On the other hand, test results are shown in a few hours and can easily be performed on a large mass of patients. Low sensitivity and stability, and a long time for the collecting & transferring of the sample are the disadvantages of this test. Several external factors can also affect the accuracy of this test, including sampling operation, sample source (upper or lower respiratory tract), sample collection time (before or after the onset of symptoms), and whether serological tests are used or not simultaneously. Recent studies have shown that commercial diagnostic kits used in the market for diagnosing SARS-CoV-2 have low diagnostic accuracy (less than 100%) and false-negative results in patients during the first week of illness have also been reported [46].

Broad studies carried out on the tests used for diagnosing coronavirus in China show that the results of 41% of cases were false negatives [47]. Besides, real-time RT-PCR testing requires trained individuals to use complex laboratory equipment correctly. On the one hand, these tests are performed in central laboratories (level two and above), on the other hand, they are time-consuming and it takes several hours to one or two days, to obtain laboratory results. This often leaves a rapidly rising number of potential cases untested and thus opening a gaping hole in SARS-CoV-2 prevention efforts. Finally, the US Food and Drug Administration (FDA) concluded that a negative RT-PCR test result does not completely rule out SARS-CoV-2 infection and shall not be used as a single element for patient management decisions, and re-testing shall be considered in consultation with public health authorities [43].

b. *Rapid & Point of care NAAT*: RT-LMAP has made it possible to perform molecular diagnostic tests in a patient-care facility instead of a laboratory setting. This method also increases the number of tests to be performed, shortens the time for announcing test results, and paves the way for early detection of positive cases [48]. LAMP (Loop-mediated isothermal amplification) is a fast, accurate, reliable, and inexpensive technology that requires only one thermal cycle to determine the genomic sequence, unlike real-time RT-PCR, which is complex and need sophisticated laboratory thermal cycling equipment. The main advantage of LAMP over real-time RT-PCR is that the amount of DNA it produces is far greater than PCR, and positive results can be seen with the naked eye, as opposed to PCR, which the machine must read the results. Also, it is simple, inexpensive, and has high-performance speed [49].

1.2.3 Chest computerized tomography

A chest scan is a routine, non-invasive, rapid, and accurate radiological test. The sensitivity of CT scan to detect Covid-19 is high, compared to real-time RT-PCR. Recent studies have shown that asymptomatic patients of Covid-19, can show bilateral chest radiographic evidence (ground-glass opacities) on CT scan even before real-time RT-PCR is positive [50]. Moreover, scientific evidence shows that the best approach to suspected patients of COVID-19 is to perform combination tests including real-time RT-PCR, epidemiological evidence (exposure to patients with Covid-19, presence of symptoms and symptoms), and chest CT scan [51].

1.2.4. Tissue culture & Neutralizing test with Actual or Pseudo Virus

Virus neutralization assay (VNA) is one of the most specific tests to study the reaction of antibodies to the virus and prevent their proliferation. This test detects only those antibodies that prevent the virus from multiplying, not all antibody reactions. Because common antigens

among viral groups can be the same, only some of these antigens are targeted by neutralizing antibodies [52]. VNA testing is performed in four steps: diluting the serum, incubating the serum with the virus, inoculating the cell culture, and identifying and detecting. Although VNA testing is highly specific, it is extremely complex, time-consuming, and requires skilled staff to perform the test. At present, VNA tests are done using microtiter plates which are relatively inexpensive and straightforward to perform using standard laboratory equipment [53].

1.2.5. Techniques under Development

Diagnostic tests that have not yet been widely used to diagnose Covid-19 and are under development are as follow:

a: CRISPR-Cas12 (Clustered Regularly Interspaced Short Palindromic Repeats): This test is one of the most sensitive, specific, fast, and simple PCR tests, by which nucleic acids are detected. This molecular method can target and detect up to 10 copies of SARS-CoV-2 nucleic acids in samples without using any special equipment. Therefore, this method is considered suitable for use in local centers and hospitals [54].

b: Gold Nanoparticles: this is one of the new technologies in the field of medicine and diagnostics. This molecular method is simple, fast, and sensitive which facilitates quantitative detection with excellent multiplexing capabilities. Gold nanoparticles were greatly envisioned as state-of-the-art technologies for rapid viral detection. But so far no evidence of their use has been reported for detection of SARS-CoV-2 [55].

c: SERS (Surface-Enhanced Raman Scattering): SERS, which uses fluorescein, has emerged as a powerful molecular analysis method. This method is one of the most sensitive techniques used to obtain multiple components in a mixture or sample. This technique can still be used as a point-of-care test. However, no studies have been reported on the use of this technology to detect SARS-CoV-2 [55].

1.3. Post analytic stage

Initially, In the United States, the CDC assay, if both targets (nucleoprotein N1 and N2) tests were positive in RT-PCR, the case was considered to be laboratory confirmed [56]. The low cycle of threshold denotes high viral loads in the specimen. Viral loads determined by RT-PCR should not be used as an indicator of the severity or to monitor therapeutic response, however, it may be used as an indication for transmissibility [13, 57]. A cycle threshold (C_T) value of less than 40 is defined as a positive test, while a C_T value of 40 or more is defined as a negative test. A C_T value of 40 for only one of the two nucleocapsid proteins (N1 and N2) is defined as indefinite and requires confirmation by retesting [56]. In China, for the assays with three targets, two or more targets must be positive to consider a case positive [58]. Monitoring of patients during recovery from COVID-19 pneumonia is very important for ending isolation. If discharged patients still shed viable viruses during this period, they can still transmit the infection to others. Therefore self-quarantine for at least one month is recommended in such situations [59]. Being infective or being cured can't be determined by OP/NP swabs alone [60], this issue needs further investigations. A person can be cured if, two consecutive negative RT-PCR has been demonstrated from rectal swabs [22, 61]. Therefore a positive rectal swab for SARS-CoV-2 with RT-PCR means that the patient shed viable viruses through his feces thereby remaining infectious [12, 18, 23-25]. However, in another study on 20 COVID-19 patients despite high viral RNA concentration, no virus was isolated from fecal specimen [8]. The

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correlation of positive RT-PCR results, with no virus isolation in the same sample needs further investigations.

2. Method and materials

2.1 Information sources and search strategy

A literature search was performed for the present study on the lines for a narrative review, but including features of systematic review methodology (PRISMA flow diagram). Three authors searched three electronic databases for literature review Google Scholar, HINARI and PubMed using keywords/terms, specimen types or molecular diagnostic tests or serological tests in link with COVID-19 or SARS-CoV-2, with different combinations, between March 21. 2020 and March 8. 2023.

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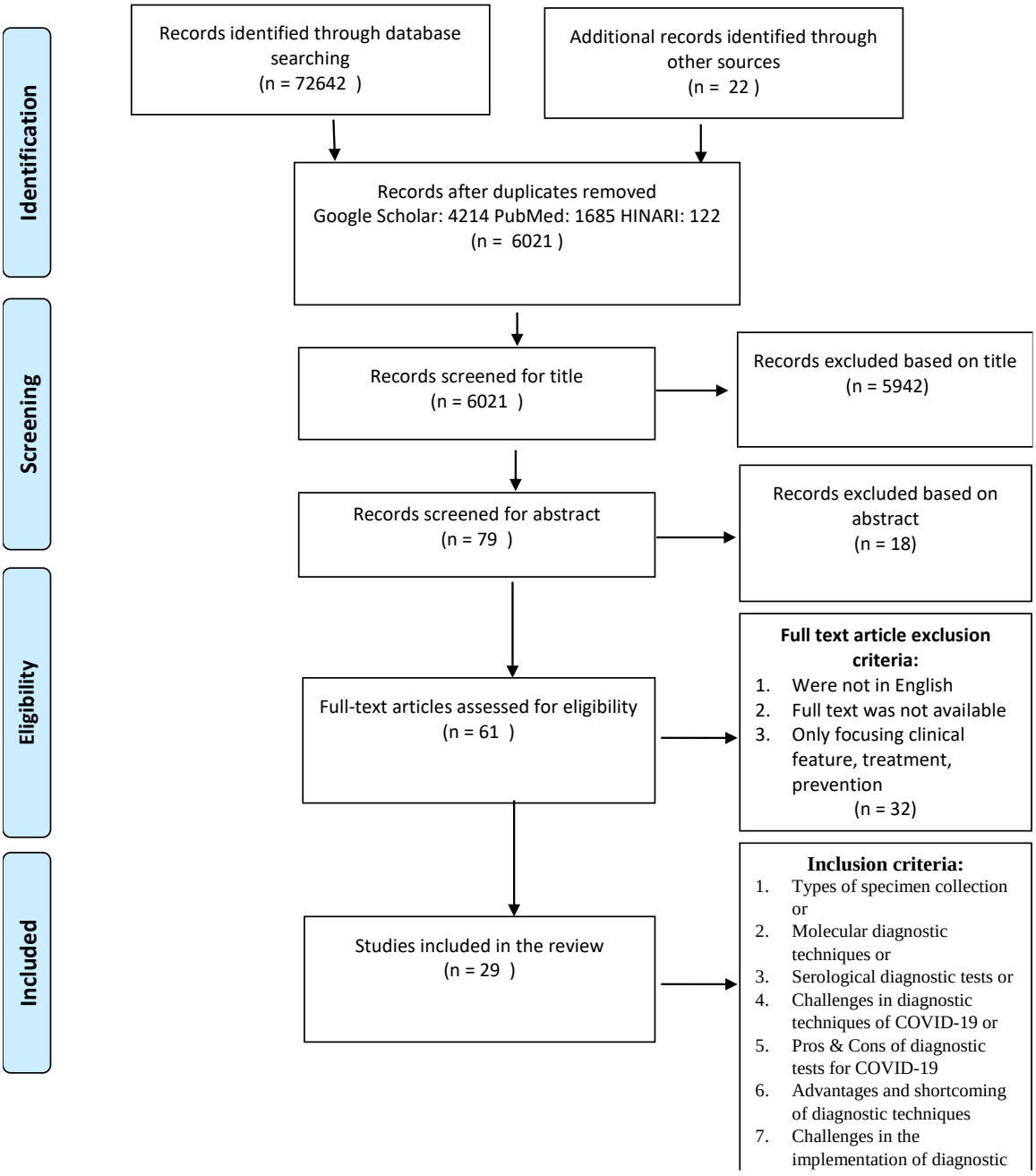
PRISMA FLOW DIAGRAM IN OUR STUDY



As depicted in the Prisma Flow Diagram, a total of 72664 records were retrieved. After removing duplicates using Endnote X8, 6021 (4214 from Google Scholar, 1685 from PubMed, 122 from HINARI) records were screened by two independent authors in titles, abstracts and full-text stages. Of the 6021 retrieved records, 5942 titles were excluded and 79 remaining records were screened for the abstracts. Of these 79 abstracts, 18 were excluded for not being relevant/ not match our research objectives. The 61 remaining records were evaluated for eligibility criteria of which 32 were excluded. The reasons for their exclusion were:

1. Articles not written in English,

2. Articles which no full-text was available for,
3. And articles which were focusing only clinical feature, treatment & prevention.

29 records fall in our including criteria for extensive reviewing, analysing and synthesis. We have included articles for full-text review only if they were focusing on one or all of the following:

1. Types of specimen collection or
2. Molecular diagnostic techniques or
3. Serological diagnostic tests or
4. Challenges in diagnostic techniques of COVID-19 or
5. Pros & Cons of diagnostic tests for COVID-19
6. Advantages and shortcoming of diagnostic techniques
7. Challenges in the implementation of diagnostic techniques

3. Summary highlights

1) The nasopharyngeal swab is the preferred specimen type for molecular diagnostic tests.
2) Inappropriate and inadequate preparation of samples (unsuitable conditions for transport and storage of samples, presence of prohibitory substances in samples, errors in pipetting during manual preparation of samples, non-compliance of samples and cross-contamination) are the major sources of preanalytical & analytic errors which can greatly jeopardize the final results.
3) Serological tests have great role in diagnosing of late infection of COVID-19, asymptomatic patients who are in convalescence period and determination of prevalence of SARS-CoV-2 in the community.
4) RT-PCR is the method of choice for various specimen types for definitive diagnosis of early infection of SARS-CoV-2.
5) CT-scan is the best approach for suspected patients of COVID-19 in the early stage of the disease in combination with rt-PCR.
6) Culture is not recommended for diagnostic purposes of SARS-CoV-2.

4. Discussion

To contain the ongoing SARS-CoV-2 pandemic it's essential to establish an early diagnosis and identification of infection and subsequently prevent the transmission of infection by making decision for isolation and properly treatment of patients. Early identification of infection needs precise and accurate molecular diagnostic tests. The final results of these diagnostic tests can be greatly influenced by proper sample collection from the right anatomical site and in the proper time. This manuscript has tried to bring in light various types of specimen collection for detection of SARS-CoV2, molecular and serological-based diagnostic techniques and challenges in their implementation.

A nasopharyngeal swab rather than oropharyngeal swab in the early stage of infection is recommended as it yielded highest viral load, is safe to the conductor & well tolerated by the patients. The combination of NP and OP swabs can enhance the sensitivity, but requires twice numbers of swabs. For epidemiological purposes and asymptomatic persons with history of no exposure, self-collected saliva or nasal wash could be used as an alternative specimen type. In the late stage of the disease or when the NP swab is negative, deep sputum or bronchoalveolar lavage (BAL) is indicated but it should be reserved for advanced infection & those patients who

are severely ill as it is an invasive procedure which need intubation and increases the risk of exposure of healthcare workers during sample collection. A recent study [12] has revealed that bronchoalveolar lavage (BAL) had the highest positive rates (93%) followed by sputum (72%), nasal swabs (63%), pharyngeal swabs (32%), feces (29%) and blood (1%). This variation is due to the fact that the viral loads of SARS-CoV-2 in respiratory specimen during the early stage of infection is higher, which than decline at week 3 to 4 to undetectable levels [10, 62, 63]. Another study has shown that the faecal specimens remained positive using RT-PCR even after the nasal specimen turned negative [20]. This suggests that faecal-oral transmission may serve as an alternative infection route for SARS-CoV-2. Transmission of the virus via pulmonary & extra pulmonary routs can help explain the rapid spread of infection. Additionally, taking of samples from different sites at different times can improve the sensitivity and decrease the false-negative test results [12].

Despite the false-negative results, molecular diagnostic tests including RT-PCR and RT-LAMP considered the gold standards for definitive diagnosis of SARS-COV-2 in various clinical specimens [64-66]. While a higher sensitivity (97%) for CT-scan rather than RT-PCR (71%) has been reported [51], CT-scan can't differentiate radiological features caused by pathogen other than SARS-CoV-2.Thus, Its efficiency is enhanced when performed in combination with RT-PCR [67, 68]. Unfortunately, the current molecular diagnostic tests are laboratory-based time-consuming, need professional employees, and human resources, and lastly, lack of adequate molecular diagnostic kits delays the diagnosis [43].

Serological tests however, have poor sensitivity for detecting early infections, they have prominent role in diagnosing positive cases, asymptomatic individuals who are in convalescence period and prevalence of infection in the community. Combination of serological and molecular diagnostic tests can provide satisfactory results and greatly decreases false-negative test results [39].

5. Conclusion

To contain the ongoing pandemic of SARS-CoV-2 and reduce the pressures on health systems around the world, the capacity of diagnostic tests should be improved. Rapid molecular diagnostic tests that can be fast, reliable, cost-effective, available, sensitive, specific & point of care, must be urgently developed, produced, and widely distributed all over the world.

6. Ethical consideration

Since this was a review paper, therefore, no ethical approval was required to conduct the study.

7. Conflict of interest

The authors declare no conflicts of interest.

8. Funding

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9. Acknowledgement

Not applicable.

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