OPEN ACCESS Freely available online

Journal of Clinical Chemistry and Laboratory Medicine

Review Article

Laboratory Diagnosis of COVID-19: Role of Laboratory Medicine

Teklehaimanot Kiros*, Mulgeta Kiros, Henock Andalem, Wasihun Hailemichael, Shewaneh Damite, Tahir Eyayu, Sisay Getu, Tegenaw Tiruneh

Department of Medical Laboratory Sciences, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

ABSTRACT

COVID-19 has still been spreading globally since its discovery. The disease has been recognized as the public health emergency of international concern. An appropriate laboratory testing as part of the critical role of laboratory medicine is providing a tremendous contribution in diagnosing and managing of medically important viral human pathogens notably SARS-CoV-2. The emergence of SARS-CoV-2 in the 21st century is posing serious health and socioeconomic threats worldwide. This clearly indicates a paradigm shift to global collaborative efforts especially for the development of novel antiviral drugs and vaccines to save lives globally. The current statistical data of the virus is indicating that the level of response against the virus is significantly low. This elucidates that the role of laboratory medicine to combat against the novel virus is very limited especially at developing countries like Ethiopia mainly due to the scarcity of the resources and manpower. Hence, assay selection for appropriate detection and genomic characterization of SARS-CoV-2 from the various biological specimens such respiratory and none respiratory specimens is considered as the frontline strategy to mitigate the rapid spreading of the virus. Despite novel testing assays with improved diagnostic accuracy, specificity and sensitivity are developing; inappropriate specimen collection procedure, improper specimen (inadequate volume and low quality), the presence of interfering substances or inhibitors have remained the major challenges to secure reliable results. Therefore, careful interpretation of laboratory results is fundamentally recommended due to the presence of such interfering errors in the pre-analytical, analytical and post-analytical phase of testing.

Keywords: SARS-CoV-2; Laboratory medicine; Coronavirus; Specimen collection; RT-PCR

INTRODUCTION

An unprecedented outbreak of 27 cases of pneumonia of unknown etiology has emerged in Wuhan City, Hubei province of China on 31st December 2019. The cluster of patients most notably presented with clinical symptoms of dry cough, dyspnea and fever. At a time, cases were all linked to Wuhan's Huanan seafood wholesale market which trades in fish and a variety of live animal including poultry and bats. Later on, the causative agent was identified from throat swab samples conducted by the Chinese Centre for Disease Control and Prevention on 7th January 2020 [1]. The novel coronavirus diseases (2019-nCoV) was officially changed to coronavirus disease 2019 (COVID-19) on February 11, 2020, by the world health organization [2]. According to the Coronaviridae study group (CSG) of the international committee on taxonomy of viruses which is responsible for the classification of viruses and taxon nomenclature of the family Coronaviridae, the tentative naming of the 2019-nCoV have changed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on phylogeny, taxonomy and established practice indicating that the virus has shown a forming a sister clade to the prototype human and bat SARS-CoV [3].

The rapid evolution of human coronavirus (HCoV) infections alarmed the global health community for the necessity of readily available, accurate and fast diagnostic testing methods to contain the fast contagiousness of the virus. Currently, many conventional and rapidly emerging laboratory testing methods for the diagnosis of HCoV infections are developing even with

Correspondence to: Teklehaimanot Kiros, Department of Medical Laboratory Sciences, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia, Tel: 251911009588; E-mail: tkt0932@gmail.com

Received: June 01, 2020; **Accepted**: June 15, 2020; **Published**: June 22, 2020

Citation: Kiros T, Kiros M, Andalem H, Hailemichael W, Damite S, Eyayu T, et al. (2020) Laboratory Diagnosis of COVID-19: Role of Laboratory Medicine. J Clin Chem Lab Med. 3:142. DOI: 10.35248/clinical-chemistry-laboratory-medicine.20.3.142

novel assays to cope up with emerging viral pathogens. This creates a better opportunity for replacing conventional methods with newer laboratory assays that are with improved diagnostic accuracy, high sensitivity and specificity [4,5]. COVID-19 is rapidly spreading from its origin of Wuhan City to the rest of the world with strange public health, social and economic crisis. Once COVID -19 is recognized as a public health emergency of international concern, appropriate laboratory diagnosis of SARS-CoV-2 together with the intervention such as social distancing, contact tracing, and hand hygiene is playing the most important role to alleviate the virus spreading. Proper collecting of the clinical specimen at the right time from the right anatomic site with the right procedure significantly offers the right treatment to the right patient at the right time. So, it is essential to establish laboratory diagnostics to accelerating the rapid, timely and accurate reporting of results to concerned bodies besides to take countermeasures, especially for the frontline staff to keep safe while working to produce reliable test results [6,7]. Furthermore, equipped laboratory settings will facilitate the rapid surveillance, monitoring and establishing robust strategies for infection prevention and control caused by SARS-CoV-2 [8]. Therefore, this review article is aimed to present:

1. Choice of specimen for laboratory testing of COVID-19
2. Safety precautions for collecting, handling, processing and storage of various clinical specimens obtained from suspected or confirmed COVID-19 patients
3. Description of the various potential errors encountered during pre-analytical, analytical and post-analytical phases of testing
4. Status updates of the diagnostic approaches available for testing of COVID-19
5. The critical role of laboratory medicine during pandemics how to interpret diagnostic tests results.

ETIOLOGIC AGENT

Corona viruses belonging to the family of Coronaviridae are the largest RNA viruses which are enveloped positive-sense RNA viruses ranging from 120-160 nm in diameter with spike-like projections on its surface giving it a crown-like an appearance under the electron microscope. The viruses have a wide range of biological host and tissue tropism including respiratory, gastrointestinal, hepatic, central nervous systems of humans, mammals and birds. The family includes three class of vertebrates: mammals (corona and to roviruses), birds (corona viruses) and fish (bafini viruses) [3,9]. The family Coronaviridae includes four genera such as Alpha coronavirus, Beta coronavirus, Delta coronavirus and Gamma coronavirus in addition to many subgenera and species. The most important HCoVs include HCoV-229E, HCV-NL63 (genus Alpha coronavirus), HCV-OC43 and HCoV-HKU1 (genus Beta coronavirus). CoV-229E and CoV-NL-63 under the genus of Alpha coronavirus cause the diseases mild respiratory tract infection, the Beta coronavirus of CoV-HKU-1 and CoV-OC43 are responsible for mild respiratory tract infection and pneumonia. Similarly, the SARS-CoV-2 β human are responsible for the severe acute respiratory syndrome [10,11]. HCoVs were first isolated in cell culture in the 1960s from persons with upper respiratory infections. HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 were isolated from persons with bronchiolitis and pneumoniain the early 2000s [12]. Beta coronavirus in lineage B of subgenus Sarbe covirus that were originated in bats then spread from civets to humans later named severe acute respiratory syndrome coronavirus (SARS-CoV) [13]. Beta coronavirus in lineage C of subgenus Merbecovirus were originated from Saudi Arabia in 2012 officially named Middle East respiratory syndromecoronavirus (MERS-CoV) [14,15]. The emergence of SARS-CoV-2 has marked the third introduction of a highly pathogenic coronavirus into the human population in the 21st century. SARS-CoV-2 uses the same receptor angiotensin-converting enzyme 2 (ACE2) similar to the previous SARS-CoV that mainly spreads through the respiratory tract. Genomic sequencing shows SARS-CoV-2 to be closely related to Beta corona viruses that detected in bats (88% sequence identity). SARS-CoV-2 is taxonomically related to the subgenus Sarbecovirus together with SARS-CoV and bat SARS-like CoV. Virions are mostly spherical, with pronounced spiked glycoprotein (S) embedded in the envelope. Additional structural proteins include envelope (E), matrix (M), and nucleocapsid (N). Intra and inter-species transmission of CoVs, and genetic recombination events contribute to the emergence of new strains of the virus [16,17].

SPECIMEN COLLECTION: GENERAL CONSIDERATIONS

Proper collection of specimens is the most critical step in the laboratory diagnosis of infectious diseases notably viral infections. Improper collection of specimen either from suspected or confirmed cases of COVID-19 patients may lead to misdiagnosis and inconclusive results. Hence, standard operating procedures (SOP) for specimen collection to all clinical laboratory are very important [4,18]. To provide physicians with the answers they need to manage patients effectively during an outbreak setting, laboratory testing based on the correct specimen is needed at the front lines. This is especially sounded during an outbreak like COVID-19. Rapid collection and testing of appropriate specimens from patients meeting the suspected case definition for COVID-19 [19] is a top priority for clinical management lead by laboratory experts to enhance outbreak control. Suspected cases should be screened for the virus either with nucleic acid amplification tests (NAAT) such as real-time polymerase chain reaction (RT-PCR) or serological tests. Strictly adhering to the safety procedures during specimen collection from suspected or confirmed COVID-19 cases should be maintained following adequate SOPs. Besides, care should be taken during storage, packaging, and shipping all specimens collected for laboratory investigations whilst they are regarded as potentially hazardous [20,21].

Specimens collected from the surface of the respiratory mucosa with nasopharyngeal swabs is one procedure used for the diagnosis of Covid-19 infection in adults and children. The procedure is also commonly used to evaluate patients with suspected respiratory infection caused by other viruses such as SARS-CoV and MERS-CoV. For SARS-CoV-2 infections, there is no specific contraindication for collecting specimens with

nasopharyngeal swabs. However, it is noteworthy that clinicians should be cautious if the patient had recent nasal trauma, surgery, markedly deviated nasal septum, history of chronically blocked nasal passages, severe coagulopathy ahead of specimen collection [7,19,22-25].Care is very important to get ride-off many potential assay vulnerabilities, particularly to the real-time reverse transcription-polymerase chain reaction (rRT-PCR). For example, to the pre-analytical aspects such as inappropriate specimen collection, handling, transport, storage, labeling or inadequate material either in quality or volume, presence of interfering substances, manual errors, as well as specific aspects such as sample contamination and testing patients receiving antiretroviral therapy. Some analytical problems may also contribute to jeopardize the diagnostic accuracy, including testing outside the diagnostic window, active viral recombination, use of inadequately validated assays, insufficient harmonization, instrument malfunctioning, along with other specific technical issues [22,25]. Some practical indications can be identified for minimizing the risk of diagnostic errors through the improvement of diagnostic accuracy by combining clinical evidence with results of RT-PCR, interpretation of RT-PCR results according to epidemiologic, clinical and radiological factors, recollection and testing of the upper (or lower) respiratory specimens in patients with negative RT-PCR test results and high suspicion or probability of infection. Furthermore, dissemination of clear instructions for specimen collection, management and storage should be pass through quality assurance measures [6,26].

SPECIMEN TYPES

Appropriate specimen collection and processing is the first and very important step for laboratory diagnosis of several infectious agents. Specimen types of patients with respiratory virus infection are diverse. Viral RNA could be detected in the upper respiratory tract (URT), lower respiratory tract (LRT), stool, blood, saliva and urine of SARS-CoV, MERS-CoV, and SARS-CoV-2 infected persons. Among them, URT specimens, as well as LRT specimens in severely affected patients, should be collected for diagnosis. Retesting if negative results in patients with either epidemiological history or suspected symptoms of SARS-CoV-2 infection is advisable [7]. Contemporary studies reported that SARS-CoV-2 could be detected in stool, blood and urine even if it negative in URT specimens. So collecting diverse samples improve positive rate especially when respiratory specimens are unavailable [27,28]. To demonstrate viral clearance during treatment, samples should also be collected and tested repeatedly. World health organization (WHO)recommended that the frequency of specimen collection should be at least every 2 to 4 days until there are two consecutive negative results in a clinically recovered patient at least 24 hours apart [29]. Also, Chinese national health commission and the US centres for disease control and prevention (CDC) recommended that negative results of rRT-PCR testing for SARS-CoV-2 from at least two sequential respiratory tract specimens collected at least 24 hours apart can be considered to discontinue transmission-based precautions [19,21,27,30]. Although SARS-CoV-2 RNA is detected from various types of the specimen [30-40], much emphasis is given for URT, LRT and serum specimen collection protocols in this review as described below.

UPPER RESPIRATORY TRACT SPECIMENS

Several points should be taken into consideration whenever determining the type of specimen and collection procedure from a patient under investigation for COVID-19. This is because the number of days between specimen collection and symptom onset as well as symptoms at the time of specimen collection is important for testing and to implement adequate infection prevention and control (IPC). Nasopharyngeal and oropharyngeal swab (NP/OP swab) collection from the URT is very critical for the diagnosis of coronavirus. But, this should be made with standard collection methods, equipment, handling, storing and shipping specimens following appropriate protocols [7,8,29,33,41]. For specimen collection of the URT, equipment like NP swabs that specifically have long, flexible shafts made of plastic or metal and tips made of polyester rayon or flocked nylon are required. In addition to NP, personal protective equipment (PPE), including a gown, gloves, protective surgical mask, face shield and safety shoe are essential components. Whenever possible, PPE should be worn in the presence of an observer to make sure there are no breaks in a technique that may pose a risk of contamination to the health personnel. The suiting procedure for PPE should be: (1) put on a protective gown, (2) wash your hands with soap and water, (3) put on a pair of surgical gloves after optimal drying,(4) put on a protective N95 mask, (5) finally put on a face shield for face and eye protection [8,29,42,43]. As standard procedure, N95 masks are strongly recommended for all patients either suspected or confirmed case of Covid-19 infection.

To collect NP/OP swab, first, ask the patient to take off her or his mask and blow her or his nose into a tissue to clear excess secretions from the nasal secretion. Secondly, remove the swab from the packaging and tilt the patient's head back slightly. This is to make the nasal passages easily accessible. Thirdly, ask politely the patient to close her or his eyes to lessen the mild discomfort of the protocol; then gently insert the swab along the nasal septum just above the floor of the nasal passage toward the nasopharynx until resistance is felt. Fourthly, insert the swab into the nostril carefully parallel to the palate. At this point, if resistance detects to the passage of the swab please try reinserting it at a different angle closer to the floor of the nasal canal carefully. The swab should reach a depth equal to the distance from the nostrils to the outer opening of the ear. Leaving the swab in place for several seconds hastens to absorb of secretions by slowly removing the swab while rotating [7,27,43,44].

It is advisable not to use calcium alginate and wooden shafts swabs. Because it may contain substances that inactivate some viruses as well as RT-PCR testing. In addition, it is strongly recommended to place swabs immediately into sterile tubes which are leak-proof, screw-cap, sterile and dry containing 2-3 ml of viral transport media (VTM).URT swabs like NP and OP should be kept either at 4°C for five days or at -70°C (dry ice) for longer than five days. If transportation of the sample is required to referral laboratory then, Dacron or flocked swabs with VTM

at 2-8 °C should be maintained [19,24,27,29,37,44,45]. Regarding the handling of the specimen, it is important to insert the swab into the tube and break the swab at the groove then, discard what remains of the swab. After, the labeled collection tube should be placed in a biohazard bag to control the spreading of the virus in the community and the medical personnel themselves [7,8,19,22]. Once completed the task careful removal of PPE is compulsory following the standards precaution. It includes: removing gown and gloves, cleaning hands with soap and water, put on a new pair of gloves, remove face shield, removes your gloves, rewashes your hands, put on another pair of gloves; and then remove your mask [11,20,43,46].

LOWER RESPIRATORY TRACTS SPECIMENS

This includes Broncho alveolar lavage (BAL), tracheal aspirate, pleural fluid and sputum. Although SARS-CoV-2 RNA could be detected among various LRT specimens, sputum is preferred by several guidelines for the laboratory diagnosis of COVID-19 infection. Dehydration may lessen the fluid in the lungs and make it hard to produce sputum so that instructing the patient to drink water before collection can increase the availability of the material [24,44]. For sputum collection procedure, instruct the patient to rinse the mouth with water prior sputum collection. Then, allow to cough deeply the sputum directly into a sterile, leak-proof, screw-cap and dry container optimally a 2-3 mL in volume. In this regard, the patient should cough deeply so that sputum rather than oral secretions are collected appropriately by health personnel. The specimen should be kept following the standard guidelines for handling and storage. At this condition, a sterile container with VTM except to sputum should be handled at 4°C for 48 hours and at minus70°Cif longer than 48 hours [19,33,37].

SERUM

Other none respiratory specimens like a serum for antibody testing is very important for epidemiological and clinical investigation. This samples may be collected at a couple of weeks or more weeks after symptom onset. The serologic testing of respiratory pathogens requires the appropriate collection and testing of paired sera [23,27]. For RT-PCR testing, a single serum collected optimally during the first 10-12 days after symptom onset is recommended. The minimum amount of serum required for SARS-CoV-2 testing either serologic or molecular assay is 200 µL. Depending age, collect for children and adults about 3-5 mL and a minimum of 1 mL for an infant. Then, keep the serum specimen at 2-8°Cand ship on icepack [8,23,46,47].

FACTORS INFLUENCING DIAGNOSTIC ACCURACY

The quality of specimens obtained from patients for testing can be affected by several factors including specimen collection protocols (quality, adequacy), transport and storage. These factors may exist in almost all biological specimens such as the upper and lower respiratory samples, body fluids, stool, urine and the likes [22,30,31,33,38,42,43]. Laboratory medicine is playing a critical role during this pandemic in diagnosing, treating, isolating and managing many important human pathologies causing several infectious diseases globally. However, the diagnostic accuracy of test results and their correct interpretation are confronted by factors occurring during the pre-analytical, analytical and post-analytical phases shown in Table 1.

Table 1: Potential interfering factors during testing of SARS-CoV-2 RNA infection.

Phases	Interfering factors	Reference
PrA	Lack of identification and misidentification of the patient	(7, 19, 20, 22, 44, 45)
	Inappropriate test procedures	
	Inadequate volume and poor quality of sample	
	Virus retention medium containing guanidine salt	
	Presence of interfering substances or testing inhibitors	
	Lack of SOP as well as safety manuals	
	Manipulation with a contaminated sample	
	Sample from patients receiving antiretroviral therapy	
	Performing test out of the standard workplace like BSC	(7, 19, 20, 22, 44, 45)
	Active viral recombination, repeated freezing or thawing	

	Lack of performing internal QC to ensure method validation	
	Lack of harmonization of primers, probes and non-specific PCR annealing	
	Instrument malfunctioning and insufficient problem shooting guideline	
	Misinterpretation of test results and poor communication of results	
	Lack of appropriate documentation of results	(7, 19, 20, 22, 44, 45)
PoA	Delay in releasing results to the clinician	

Abbreviation: PrA (Pre-analytical), A (analytical), PoA (post-analytical), QC (quality control), SOP (standard operating procedure), PCR (polymerase chain reaction), BSC (biosafety cabinet)

As a result, current gold standard assays like rRT-PCR for diagnosing of many infectious diseases as well as the current pandemic COVID-19 needs calibration and validation for the presence of any potential vulnerabilities on the occupation of either rRT-PCR or serological testing for SARS-CoV-2 infection [10,22,29,42].

THE DETECTION RATE OF SARS-COV-2 FROM VARIOUS SPECIMENS

SARS-CoV-2 could be detected from diverse clinical specimens like URT, LRT, blood, urine, stool, and saliva. The diagnostic sensitivity and specificity of the virus significantly vary depending on the source of the specimen used for testing [23,42,48]. So investigation of SARS-CoV-2 among the different clinical specimen in Table 2 is the most critical step to promote a high quality of testing, treatment and infection containment [23,30]. The timeline of test positivity varies among specimens. For example, PCR positivity declines more slowly in sputum but still be positive after NP swabs were reported negative. Moreover, PCR positivity in a stool specimen was detected around 57% of infected patients beyond NP swab by a median of 4 to 11 days despite correlation to the clinical severity of the disease remain obscured [49]. An investigation by Wölfel, [50] showed that the persistence of PCR in sputum and stool was found to be similar. But, another study [36] of 205 patients with confirmed COVID-19 infection found that highest in BAL(93%), followed by sputum (72%), nasal swab (63%), and pharyngeal swab (32%). A study on the viral nucleic acid detection of SARS-CoV-2 among 52 cases using throat swab and sputum specimen indicated that significantly higher positive rate detection from sputum (79.9%) than throat swab that can facilitate the selection of specimens to ensure the accuracy of diagnosis [33].

Table 2: Detection rate of SARS-CoV-2 RNA according to the type of specimens.

Types of specimens	Detection rate (%)	References
Bronchoalveolar lavage fluid	>90	(7, 22, 36)
Saliva	13-89	(7, 35, 36, 38)
Sputum	48-77	(7, 14, 33, 36, 39, 47)
Nasopharyngeal swab	Jun-80	(3, 7, 36-39, 41)
Oropharyngeal swabs	Apr-70	(7, 34-37)
Stool	Oct-60	(6, 7, 31, 34, 36, 39, 49)
Throat washing	30-44	(7, 25, 33, 47)
Blood	Jan-50	(6, 30, 34, 39, 44, 47)
Urine	Apr-37	(7, 34, 44)

BIOSAFETY MEASURES

According to WHO laboratory biosafety guidance regarding COVID-19 [51], it is essential to adhere strictly to standard laboratory biosafety practices when clinical staff are performing with specimens took from suspected and confirmed cases. For example, initial processing of all specimens should be performed under a validated biological safety cabinet (BSC). Non-propagative diagnostic laboratory work such as the NAAT and propagative work like viral cultivation should be conducted at BSC-2 and BSc-3 levels respectively [19,46]. For this reason, biosafety measures are strongly recommended while working with high-risk infectious agents. Developing biosafety guidelines while conducting with specimens from suspected or confirmed SARS-CoV-2 patients is the epicentre of laboratory testing as its top priority of concern [8,48,52]. CDC interim laboratory biosafety guidelines also recommend strict adherence to the universal safety precautions when handling clinical specimens which are considered potentially dangerous [53].

LABORATORY TESTING METHODS

Effective isolation of the etiologic agent causing COVID-19 infection from different clinical specimens [36,39] requires a virus-specific testing protocol made by specific molecular tests and emerging serological tests [40]. However; approach to differential diagnosis from the most common types of respiratory viral infections such as Influenza, Parainfluenza,

Respiratory Syncytial virus, Adenovirus, human metapneumovirus and bacterial infections like mycoplasma, chlamydia and related disease is the most critical step to hasten timely diagnosis and prompt accurate treatment for the patient under investigation (PUI). So far, the golden clinical diagnosis method is nucleic acid detection by rRT-PCR [16,18]. According to WHO [29] for better medical discretion, clinical and epidemiological factors should be evaluated carefully along with the PCR testing of asymptomatic or mildly symptomatic contacts to suppress the viral spreading so that quick collection and testing of appropriate specimens from patients suspected for COVID-19 will be a top priority for clinical management and IPC. Because testing is the most critical to accurately detect an infectious agent which enableto generate a substantial strategy to combat against infections. Furthermore, understanding the complete nature of the test with respect to the choice of the specimen is also a key to set a high priority of testing [6,10,12,21].In this review, the current approaches for the diagnosis of COVID-19 infection encompasses molecular, serological and viral culture methods as discussed below.

MOLECULAR METHOD

Molecular testing techniques are more suitable than other imaging techniques such as computed tomography (CT) and x-ray for precise and accurate diagnoses of infections because they can target and identify specific markers of pathogens. However, use of molecular techniques requires an understanding of parameters like the proteomic and genomic composition of the pathogen and the induction of changes in the expression of proteins/genes in the host during and after infection [54]. The definitive diagnosis of SARS-CoV-2 infection is well endorsed by both the WHO [29] and CDC saying that the diagnostic testing should encompass the use of rRT-PCR assays targeting one or more genes in the SARS-CoV-2 genome using the either URT or LRT properly collected samples. Nucleic acid testing (NAT) is the primary technique for diagnosing COVID-19 infection because numerous rRT-PCR SARS-CoV-2 RNA specific kits are well designed genetically. The procedure involves detecting, sequencing, RNA isolation, purification, reverse transcription to cDNA, cDNA amplification using RT-PCR machines supported with the fluorescent signal detection [27]. According to the study [55] the *RdRP* gene (RNA-dependent RNA polymerase gene) in the open reading frame ORF1ab region, the *E* gene (envelope protein gene), and the *N* gene (nucleocapsid protein gene) are the most important genes for sequence alignment of primer design, probe selection and test optimization(reagent conditions, incubation times, and temperatures. Among the three genes both *RdRP* and *E* genes had a high analytical performance for detection with a technical limit of detection of 3.6 and 3.9 copies per reaction while the *N* gene provided poorer analytical performance or sensitivity of 8.3 copies per reaction. In this situation, the primers and probes for detecting SARS-CoV-2 antigens have been identified from genetic regions belonging to N gene involving the usage of two primer sets. It is noteworthy that molecular techniques enable the diagnosis regardless of the course of infection and any co-infection caused by other microorganisms [55].

Reverse transcription and PCR amplification at one reaction generate rapid and precise results for high-throughput analysis over a separate analysis [54,56]. The clinical application of NAT could be used either genetic heterogeneity for HCoVs with a single pan-HCoV molecular assay by using degenerate primers or utilizing multiple primer sets [45].Similarly, there is evidence others employ a single set of non-degenerate primers. The most common HCoVs (HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E) require multiple sets of PCR oligonucleotides to detect from the various clinical specimens [19,20,23,26,40,48,49].

Predominant molecular methods for diagnosing COVID-19 can be made using the URT and LRT samples. The detection of the viral load depends on the choice of the specimen and the illness onset. For example, in the first couple of weeks after illness onset, HCoVs (HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E predominantly detected in sputum followed by nasal swabs; while throat swabs were unreliable a week after symptom onset [40,45,54]. This is because of the diagnostic accuracy in Table 1 and reliability of RT-PCR heavily depends on several factors [43,49]. A study [36] showed that the rate of RT-PCR detection of SARS-CoV-2 in patients diagnosed with COVID-19 is as high as 93% in BAL fluid but then decreases to 72% in sputum and 63% in nasal swabs respectively. On the converse, it is only 32% in pharyngeal swabs and 29% in the stool respectively. This may suggest variability in the viral loads among different samples are the results of low viral load in the area sampled, mutations in the viral genome and technical problems [28,54]. Negative test results from respiratory samples but due to much epidemiological suspicion [35] have shown that the positive rate of SARS-CoV-2 is 15-30% in blood and 14-38% in rectal swabs respectively.

Chinese national health commission guideline [57] criteria for hospital discharge stated that a 2 consecutively negative RT-PCR test results separated by at least 1 day. However, a current study [58] among four patients with COVID-19 who met criteria for hospital discharge reported positive RT-PCR test results from throat swab within 5 to 13 days after discharge of admission; suggesting that recovered patients still carry a virus. Moreover, the viral RNA of throat swabs became negative but the viral-specific SARS-CoV-2 RNA antibodies (IgM and IgG targeting at S and N proteins) were produced during the recovery period [35,38,40,49,58]. Due to the presence of numerous coronaviruses [3,8,40,54] that cause respiratory and intestinal infections in humans, animals and birds, target selection for manipulation of structural proteins namely spike(S), envelope (E), transmembrane (M), helicase (Hel)and nucleocapsid (N) are very essential. For these encode structural proteins, species-specific accessory genes including RNA-dependent RNA polymerase (RdRp), hemagglutinin -esterase (HE), and open reading frames ORF1a and ORF1b are important [2,16,17,19,44,55]. Importantly, the CDC [59] recommends two nucleocapsid protein targets (N1 and N2) while WHO recommends first-line screening with the E gene assay followed by a confirmatory assay using the RdRp gene [20].

Knowledge toward a clear understanding of the nature of the diagnostic tests, interpretation of their findings and their

clinical correlation is a top priority for appropriate treatment of COVID-19 infection during pandemics. A viral RNA of the symptomatic patient indicated that viral RNA in the NP swab as measured by the cycle threshold (Ct) becomes detectable as early as day one of symptoms and reached peaks within the first week of symptom onset. A Ct value of less than 40 is clinically reported as PCR positive. Importantly, a positive RT-PCR result reflects solely the detection of viral RNA in the clinical sample regardless of distinction for viability and lability [26,40,58,60,61]. The routine confirmation of cases of COVID-19 based on WHO and CDC rRT-PCR method for detection of unique sequences of the virus encompasses viral targeted like N, E, S and RdRP genes [20,50,59]. In general, cautions for the existence of the possible causes of false results including inadequate specimen quality and volume, specimens collected too early or too late for viral detection, specimens improperly collected, handled or transported, the occurrence of viral genetic mutation, presence of PCR inhibitors and antiviral administration before testing should be necessary assessed to improve the quality of diagnostic accuracy [22,27,54,60,61].

SEROLOGICAL METHOD

Serological testing is another diagnostic method used for assessing the presence of an immune response against an infectious agent. That means serological techniques are playing a key role in COVID-19 infection that can detect indirectly by measuring the host immune response for infection ranging from mild to moderate illness. However, this type of testing is not meant to replace the identification of viral RNA for etiological diagnosis of COVID-19, rather for establishing as to whether individuals have been infected by the virus and/or have developed an immune response. So the detection of the different classes of immunoglobulins particularly IgA, IgM and IgG against SARS-CoV-2 have paramount importance for establishing whether a person has been infected by SARS-CoV-2, and has then developed antibodies against the virus [6,44,60,62]. The test has a significant role to understand the extent of COVID-19 in the community and to identify individuals who are immune and potentially "protected" from becoming infected. Several published literature indicated that antibodies begin to increase from the second week of symptom onset and found to be positive even as early as the fourth day after symptom onset; but higher levels occur in the second and third week of illness [6,20,28,54]. A study [35] unveiled that the IgM and IgG sero conversion observed at the third and fourth week of clinical illness onset; while [63] indicated that IgM begins to decline and reaches lower levels by week 5 and almost disappears by week 7; whereas another study revealed that IgG persists beyond week 7 [64]. A study showed that [65] have shown that the median time of antibodies appearance in serum begins 3-6 days after the onset of symptoms for both IgM and IgA;but delayed to 10-18 days for IgG. The positive rate of detection for the diverse classes of antibodies is 85.4%, 92.7% and 77.9% for IgM, IgA and IgG respectively. In most of the cases, the production of immunoglobulins such as IgM and IgG tend to appear 6-7 days after symptoms onset. Majority of the COVID-19 patients mount anti-SARS-CoV-2IgGnearly two weeks later the onset of symptoms [66]. Anti-SARS-CoV-2 antibody positivity up to two weeks after the onset of symptoms is as high as 100% for both IgA and IgM [40,67]. A study [68] also showed that positivity for anti-SARS-CoV-2 IgM and IgG antibodies is 50% and 95%, respectively. Similarly, a study showed that the rate of detectable anti-SARS-CoV-2 IgM and IgG antibodies in convalescent patients is 78% and 100% respectively [69]. Another investigation [70] also finds out that that the cumulative rate of positivity for anti-SARS-CoV-2 IgM and IgG antibodies 15 days from symptom onset is about 74% and 97%, respectively. In future studies, it is very important to make clear about corona virus disease whether anti-SARS-CoV-2 antibodies are neutralizing [71]. A clear understanding of the possible cross-reaction of current anti-SARS-CoV-2 immunoassays with previous corona viruses such as SARS-CoV-1, MERS-CoV, HCoV-HKU1, HCoV-OC43, HCoV-NL63 and HCoV229E is important [72].

CELL CULTURE METHOD

Cell culture is not routinely recommended for the isolation of HCoVs mainly due to lack of permissive cell lines, time-consuming, labour-intensive procedure, and expertise requirements and finally the lack of commercial antisera for culture confirmation. Cell culture was long considered the "gold standard" for virus isolation and characterization before the availability of molecular methods. Modification of cell lines including primary lines, immortalized lines, mixed cell lines, and transfected lines have played a great role in detecting and identification of infectious agents [8,44,48]. SARS-CoV-2 will grow easily in primary monkey cells and cell lines such as Vero and LLCMK2; despite the serious concern of safety issues. Viral cultivation essentially supports vaccine development and therapeutic agents [8,52,73-79].

CONCLUSION

COVID-19 caused by SARS-CoV-2 has remained a global health emergency of an international concern posing serious health, economic and social crisis. The early detection and characterization of the viral RNA from the various clinical specimen such as URT,LRT, blood, urine, stool and saliva using standard conventional and novel emerging techniques will generate a great role in timely and rapid containing of the virus. Detection of anti-SARS-CoV-2 RNA antibodies using serological methods at various weeks after the onset of illness will aid the clinical and epidemiological investigation of the virus; especially under the condition when RT-PCR is negative for a highly suspicious patient. However, information is required to validate the accuracy and reliability of this portable immunoassays. During pandemics like the current threat of COVID-19, the role of laboratory medicine is irreplaceable. The quality of many testing and result interpretation may be influenced by several factors. So establishing countermeasures to unlock these errors so that ensuring a valid, reliable and highly impactful results will be a top critical priority of laboratory medicine.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All the available data and material used in this study is presented in the main paper/manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Authors' contribution

TK was the primary researcher, conceived the study, designed, participated in data extraction, drafted and finalized the manuscript for publication. MK, HA, WH, SD, SG,TE and TT assisted in data extraction and reviewed the initial and final drafts of the manuscript. All the authors reviewed the initial andfinal drafts of the manuscript. All authors read the final version of the manuscript and approved for publication.

ACKNOWLEDGEMENTS

We authors thank our friends in the department of medical laboratory sciences for their unreserved assistance.

REFERENCES

1. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. The Lancet.2020;395:507-513.
2. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020.
3. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020:1.
4. Dai W, Zhang H, Yu J, Xu H, Chen H, Luo S, et al. CT imaging and differential diagnosis of COVID-19. Can Assoc Radiol J. 2020;71:195-200.
5. Liu W, Zhang Q, Chen J, Xiang R, Song H, Shu S, et al. Detection of Covid-19 in children in early January 2020 in Wuhan, China. N Engl J Med.2020;382:1370-1371.
6. Lippi G, Mattiuzzi C, Bovo C, Plebani M. Current Laboratory Diagnostics Of Coronavirus Disease 2019 (COVID-19).
7. Azman M, Gendeh HS, Sai Guan L, Baki MM. Upper respiratory tract sampling in COVID-19.
8. Charlton CL, Babady E, Ginocchio CC, Hatchette TF, Jerris RC, Li Y, et al. Practical guidance for clinical microbiology laboratories: viruses causing acute respiratory tract infections. Clinical microbiology reviews.2018;32:42-18.
9. Jiang S, Shi Z, Shu Y, Song J, Gao GF, Tan W, et al. A distinct name is needed for the new coronavirus. Lancet.2020.
10. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. Jama. 2020;323:1239-1242.
11. Meo S, Alhowikan A, Al-Khlaiwi T, Meo I, Halepoto D, Iqbal M, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. Eur Rev Med Pharmacol Sci.2020;24:2012-2019.
12. Su S, Wong G, Shi W, Liu J, Lai AC, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in microbiology.2016;24:490-502.
13. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med.2003;348:1953-1966.
14. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis.2013;13:752-761.
15. Guarner J. Three emerging coronaviruses in two decades: the story of SARS, MERS, and now COVID-19. Oxford University Press US.2020.
16. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Milit Med Res.2020;7:1-10.
17. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med.2020;26:450-452.
18. Singhal T. A review of coronavirus disease-2019 (COVID-19). Ind J Pediatr.2020:1-6.
19. Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, et al. Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. Ann Lab Med.2020;40:351-360.
20. Organization WH. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance, 19 March 2020. World Health Organization; 2020.
21. Sharfstein JM, Becker SJ, Mello MM. Diagnostic testing for the novel coronavirus. Jama.2020.
22. Lippi G, Simundic AM, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clinl Chem Lab Med.2020;1.
23. Chan PK, To WK, Ng KC, Lam RK, Ng TK, Chan RC, et al. Laboratory diagnosis of SARS. Emerg Infect Dis.2004;10:825.
24. Control CfD, Prevention. Interim guidelines for collecting, handling, and testing clinical specimens from patients under investigation (PUIs) for Middle East respiratory syndrome coronavirus (MERS-CoV)-Version 2.1. Atlanta, GA, USA. 2015.
25. Di Gennaro F, Pizzol D, Marotta C, Antunes M, Racalbuto V, Veronese N, et al. Coronavirus Diseases (COVID-19) Current Status and Future Perspectives: A Narrative Review. Intern J Environ Res Pub Health.2020;17:2690.
26. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clinical Chemistry and Laboratory Medicine (CCLM).2020;1.
27. Control CfD, Prevention. Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19).2020.

28. Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, et al. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (COVID-19). J Microbiol Biotechnol.2020;30:313-324.
29. Organization WH. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization; 2020.
30. Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microb Infect.2020;9:469-473.
31. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS - CoV - 2 RNA in feces of COVID - 19 patients. Journal of Medical Virology.2020.
32. Li Y, Hu Y, Yu Y, Zhang X, Li B, Wu J, et al. Positive result of Sars-Cov-2 in faeces and sputum from discharged patient with COVID - 19 in Yiwu, China. Journal of Medical Virology.2020.
33. Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). Clinical Chemistry and Laboratory Medicine (CCLM).2020;1.
34. Peng L, Liu J, Xu W, Luo Q, Chen D, Lei Z, et al. SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. J Med Virol.2020.
35. To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis.2020.
36. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. Jama.2020.
37. Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. Intern J Infect Dis.2020.
38. Williams E, Bond K, Zhang B, Putland M, Williamson DA. Saliva as a non-invasive specimen for detection of SARS-CoV-2. Journal of Clinical Microbiology. 2020.
39. Wu J, Liu J, Li S, Peng Z, Xiao Z, Wang X, et al. Detection and analysis of nucleic acid in various biological samples of COVID-19 patients. Trav Med Infect Dis.2020:101673.
40. 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. Emerg Microbes Infect.2020;9:386-389.
41. Sung RY, Chan PK, Choi KC, Yeung AC, Li AM, Tang JW, et al. Comparative study of nasopharyngeal aspirate and nasal swab specimens for diagnosis of acute viral respiratory infection. J Clin Microbiol.2008;46:3073-3076.
42. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. Med Rxiv.2020.
43. Marty F, Chen K, Verrill K. How to Obtain a Nasopharyngeal Swab Specimen. New Engl J Med.2020.
44. Yan Y, Chang L, Wang L. Laboratory testing of SARS - CoV, MERS - CoV, and SARS - CoV - 2 (2019 - nCoV): Current status, challenges, and countermeasures. Rev Med Virol. 2020:2106.
45. Tang Y-W, Schmitz JE, Persing DH, Stratton CW. The laboratory diagnosis of COVID-19 infection: current issues and challenges. J Clin Microbiol.2020.
46. Lee H, Ki CS, Sung H, Kim S, Seong MW, Yong D, et al. Guidelines for the laboratory diagnosis of Middle East respiratory syndrome coronavirus in Korea. Infect Chemo.2016;48:61-69.
47. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis.2020.
48. Loeffelholz MJ, Tang Y-W. Laboratory diagnosis of emerging human coronavirus infections—the state of the art. Emerg Microb Infect.2020:1-26.
49. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ (Clinical research ed). 2020;369:1443.
50. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature.2020.
51. Organization. WHO. Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19): interim guidance, 12 February 2020. World Health Organization.2020.
52. Mourya DT, Sapkal G, Yadav PD, SK MB, Shete A, Gupta N. Biorisk assessment for infrastructure & biosafety requirements for the laboratories providing coronavirus SARS-CoV-2/(COVID-19) diagnosis. Indian J Med Res.2020;151:172-176.
53. PG. S. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).
54. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: The Disease and Tools for Detection. ACS nano.2020;14:3822-3835.
55. 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. Euro surveillance : bulletin Europeen sur les maladies transmissibles. Europ Commun Dise Bullet.2020;25.
56. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. BioTech.2005;39:75-85.
57. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020;7(1):4.
58. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. Jama. 2020;323:1502-1503.
59. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First Case of 2019 Novel Coronavirus in the United States. N Engl J Med.2020;382:929-936.
60. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. Jama.2020.
61. Xiao AT, Tong YX, Zhang S. False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence. J Med Virol.2020.
62. Fierz W. Basic problems of serological laboratory diagnosis. Methods Mol Med.2004;94:393-427.
63. Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, et al. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.2020.
64. Xiao AT, Gao C, Zhang S. Profile of specific antibodies to SARS-CoV-2: The first report. J Infect.2020.
65. 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 : an official publication of the Infectious Diseases Society of America.2020.
66. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for

SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med.2020.

67. Lippi G, Salvagno GL, Pegoraro M, Militello V, Caloi C, Peretti A, et al. Assessment of immune response to SARS-CoV-2 with fully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays. Clin Chem Lab Med.2020.
68. Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. International journal of infectious diseases : IJID : official publication of the- Intern Soc Infect Dis.2020;94:49-52.
69. Du Z, Zhu F, Guo F, Yang B, Wang T. Detection of antibodies against SARS-CoV-2 in patients with COVID-19. J Med Virol. 2020.
70. Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect.2020.
71. Béné MC, de Carvalho M, Eveillard M, Lebri Y. Good IgA bad IgG in SARS-CoV-2 infection? Clinical infectious diseases : an official publication of the- Infect Dise Soc Am.2020.
72. Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro surveillance : bulletin Europeen sur les maladies transmissibles = Europ Commun Dis Bullet.2020;25.
73. Blow JA, Dohm DJ, Negley DL, Mores CN. Virus inactivation by nucleic acid extraction reagents. J Virol Meth.2004;119:195-198.