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Review Article

Laboratory Diagnosis of Severe Acute Respiratory Syndrome-Coronavirus-2 Infection [SARS-CoV-2, (COVID-19)]

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Abstract

Coronavirus Disease 2019 (COVID-19) is the third life-threatening pandemic disease. It started in December 2019 in Wuhan, China. Cases of SARS-CoV-2 infection may be asymptomatic or have a range of symptoms with the most common being fever, cough, and shortness of breath. Correct clinical laboratory analyses which give results in a well-timed or prompt manner are critical for the clinical and public health administration of COVID-19. The clinical presentation and stage of the COVID-19 disease determine the option as to which laboratory analysis to apply for the diagnosis. The application of the real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR), for nucleic acid analyses, is the most correct method for diagnosing acute SARS CoV-2 infection. The ideal specimen types are combined deep nasal or nasopharyngeal and throat swabs. Application of serology can be performed for the diagnosis of a previous infection that is more than 14 days after the beginning of clinical features. Antigen analyses are also carried out in most developed countries. The analytical results interpretation must take into consideration the pre-test likelihood or possibility of the patient having COVID-19 disease.

Keywords: Antibody, antigen analyses, COVID-19, nucleic acid tests, SARS-CoV-2.

INTRODUCTION

Coronavirus (CoV) are RNA viruses. They are the largest group of viruses and are members of the coronvirinae subfamily, coronaviridae family, and Nidovirales order. They are named "Coronavirus" because they have spike-like projections. These are bulbous spikes like the pointy end of a crown. Three global outbreaks in the last 20 years have been caused by coronaviruses (CoVs). The latest is coronavirus disease-19 (COVID-19). This is viral pneumonia and multi-systemic disease, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the beginning of 2020, the COVID-19 pandemic has spread globally with about 74.5 million confirmed cases and more than 1.6 million have died from the infection in 216 affected countries as of December 21, 2020 [1]. In some countries, the incidence is still rising and is well into the second or third wave of the pandemic.

The first epidemic of the twenty-first century was first reported in November 2002 in Guangdong, China. This was the first Severe Acute Respiratory Syndrome (SARS) caused by SARS-CoV (SARS-CoV-1) [2, 3]. The second outbreak termed Middle East Respiratory Syndrome (MERS) was first reported in Saudi Arabia in 2012 was caused by MERS-CoV [2, 4]. SARS-CoV-2 infection is the third and most recent outbreak (COVID-19) [3].

Because of the possibility of rapid human-to-human transmission and lack of specific therapeutic management, prompt, and consistent diagnostic analyses are very important. To prevent the spread of the virus and optimize infection control measures, reliable, accurate, and prompt analyses are necessary. Thus, reliable, prompt, and accurate diagnoses are one of the most effective strategies. The important part of decreasing the transmission, morbidity, and mortality from COVID-19 are detecting infected persons, tracing their contacts, and quarantine and isolation measures. It is generally clear that countries that test more have lower mortality rates [5-7]. The aim of this review is to

discuss the various methods of laboratory diagnosis of SARS CoV-2 infection (COVID-19).

For the screening and confirming the diagnosis of COVID-19 disease a number of analytical methods include:

a. Analyzing for nucleic acid
b. Antibody detection by serological analyses
c. Analyses for antigen

These methods may be laboratory-based or point-of-care analyses

It is very important or vital to have a better understanding of:

a. The different analytical investigations
b. Time of application
c. Type of specimens to be collected; and
d. Interpretation of test results

The SARS-CoV-2 Virus

This is a member of the coronavirus family. The coronaviruses are large, enveloped, positive-sense single-stranded RNA viruses. Human coronavirus infections are caused by α- and β-coronaviruses (8, 9). Other subfamily members include:

a. δ-coronavirus [delta-coronavirus, (delta-CoV)]
b. γ-coronavirus [gamma-coronavirus, (gamma-CoV)]

The primary hosts for α- and β-coronaviruses are bats and rodents while the primary hosts for γ- and δ-coronaviruses are birds. Like other β-coronaviruses, the COVID-19 virus has a nucleocapsid made up of genomic RNA and phosphorylated Nucleocapsid (N) protein. The Nucleocapsid is covered inside the phospholipid bilayers and enclosed by two different types of spike proteins – the spike (S) glycoprotein trimer and the haemagglutinin-esterase [10]. The membrane protein and the envelope membrane are positioned among the spike glycoproteins in the viral protein [10, 11].

The most common targets for diagnostic investigations are these four important structural proteins and these are:

a. Spike (S) protein which permits entry into the cell – attaches to receptors on target cells by the glycoprotein spikes on the viral envelope. The viral particle is then internalized by absorptive endocytosis
b. Nucleocapsid protein (N) which surrounds the genomic RNA
c. The membranous protein
d. The protein of the envelope [10, 11]

Transmission

SARS-CoV-2 is transmitted primarily through respiratory droplets or fomites [i.e. objects or material such as clothes, utensils, and furniture, which are likely to carry the virus by an infected individual (i.e. contact transmission)], and potential in faecal-oral [2, 12]. Primary viral replication is presumed to occur in the mucosal epithelium of the upper respiratory tract (nasal cavity and pharynx), with further multiplication in the lower respiratory tract and gastrointestinal mucosa. This gives rise to mild viraemia [13]. Viral shedding is thought to hit the highest point on or just before the onset of symptoms, with viral loads declining after that [13].

Receptor interaction and Cell entry

The functional receptor used by the SARS-CoV-2 virus for cell entry is the angiotensin-converting enzyme 2 receptors [12-15]. ACE2 is a member of the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases that is homologous (i.e. similar in structure) to human angiotensin 1-converting enzyme. The expression and distribution of ACE2 indicate that it might have critical roles in the regulation of cardiovascular and renal function [15, 16]. The virus binds to ACE2 via the receptor-binding domain (RBD) of the spike protein. This initiates membrane fusion and viral entry into the cell then occurs [17].

The angiotensin-converting enzyme2 (ACE2) receptor is a membrane protein that occurs in the lung, heart, kidney, and intestine and it is linked with cardiovascular disease [15]. It is also expressed in the nasal mucosa, bronchus, oesophagus, stomach, and bladder, and these organs are all vulnerable to SARS-CoV-2 infection.

Clinical Features

The transmission period for infection with SARS-CoV-2 ranges from 2 to 14 days with a mean incubation period of five days. The most common signs and symptoms are fever or pyrexia, dry cough, myalgia or fatigue, cough with a sore throat, rhinorrhoea, and anosmia (i.e. absence of the sense of smell). Sputum production, headache, haemoptysis, and diarrhoea are less common signs and symptoms [2, 16]. Dyspnoea is seen in more than half of the cases with COVID-19 disease. Cases may show normal or low white blood cell count and lymphopaenia in blood test [2].

Subjects Involved with the Analyses

The criteria for laboratory investigations or analyses include an epidemiological and clinical indication (i.e. appearance of signs and symptoms as stated above) [18]. Epidemiological indication for analyses is defined as:

a. Those in close contact with diagnosed COVID-19 patient
b. Those traveling from a COVID-19-affected part or region of the country within 14 days to the beginning of symptoms (See below)

The clinical indications for laboratory analyses or tests include the appearance of:

a. Pyrexia or fever and/or upper or lower respiratory symptoms
b. Cough
c. Pain or discomfort in the Chest
d. Breathing difficulties or shortness of breath
e. Gastrointestinal tract (GIT) signs and symptoms which include:
   i. Abdominal discomfortii.
   ii. Nausea
   iii. Vomiting
   iv. Diarrhoea, etc. [18]

In summary, the following subjects are to be tested. These are:

a. Patients that have compatible diseases
b. Asymptomatic subjects in high-risk settings, for example,
   i. Returned travelers
   ii. Healthcare workers including medical doctors
   iii. Contacts of confirmed cases – (e.g. family members of patients)
   iv. Individual in outbreak situations
c. People in enclosed environments with an increased risk of transmission, for example,
   i. Aged-care facilities
   ii. Abattoirs
   iii. Prisons

The testing guidelines can be checked and updated regularly by the Center for Disease Control (CDC) [10].

Diagnosis

Nucleic acid Analyses or Tests. The most important tests for the diagnosis of COVID-19 are viral diagnostics [2, 10]. These work by detecting SARS-CoV-2 RNA in respiratory tract samples. Active cases can be detected by the nucleic acid amplification tests (NAAT), for example, the quantitative reverse-transcription polymerase chain reaction (RT-qPCR) analyses [18]. The polymerase chain reaction (PCR) is a very sensitive and specific investigative technique for the amplification and identification of deoxyribonucleic acid (DNA). The genome of coronaviruses is made up of ribonucleic acid (RNA) and not DNA. The Taq polymerase enzyme applied for DNA amplification is not used for RNA even though it is similar to DNA. Thus, a modification of PCR analysis, known as reverse transcription-polymerase chain reaction (RT-PCR) is applied for the detection of RNA.

The reverse transcription-polymerase chain reaction is a two-step technique consisting of two enzymes – the first step applies an RNA-dependent DNA polymerase, which is also termed a reverse transcriptase, to copy RNA to DNA (cDNA), the second step then changes to the application of Taq polymerase that amplifies the cDNA as in standard PCR analysis.

Two or more RNA target sequences are normally applied. These are the gold standard (19, 20). The clinical interpretation depends on the prevalence of SARS-CoV-2 in the population analyzed. The analytical sensitivity is very high. The RT-PCR is also valuable for monitoring viral RNA shedding dynamics during the acute stage of the disease and viral RNA disintegration and disappearance during the period of recovery from COVID-19.

Other factors that contribute to the variability include:

a. Specimen collected, common specimens generally collected include:
   i. Sputum – that is material coughed up from the respiratory tract. It gives significant information affecting the diagnosis and therapeutic management of the respiratory clinical condition
   ii. Nasopharyngeal swab
   iii. Nasopharyngeal aspirate
   iv. Bronchoalveolar lavage
   v. Throat or oropharyngeal swab
b. Time of specimen collection in relation to the likelihood of viral shedding
c. Stage of the disease

During the first 5 days of the disease viral load is quite low, thus a negative sample during this stage or phase does not rule out a diagnosis. In addition, the lower respiratory tract (sputum, tracheal aspirate, and bronchoalveolar lavage) specimens have a higher viral load than those of the upper respiratory tract (nasal, pharyngeal, and nasopharyngeal). In most cases, a single negative result is usually sufficient to exclude disease, but in cases with a clinically similar disease and an elevated indicator or sign of suspicion, repeat analysis should be carried out. In general, the possibility or probability of false positives and false negatives result is very rare.

Specimen Collection for Nucleic Acid analyses or tests

The oropharyngeal (throat) sample collection is applied in order to maximize the chance of virus detection. The nasopharyngeal (i.e. bilateral deep nasal) is also suggested. By applying foam swabs for both is favored. The nasopharyngeal or bilateral deep nasal method is more sensitive than throat-only swabs. Sputum or bronchoalveolar lavage samples (on intubated patients) are preferred for cases with lower respiratory tract symptoms. Efforts to enhance testing (and perhaps including self-testing) involve drive-through methods, which consist of both self-sampling and healthcare-collected specimens [21-24].

Appropriate alternatives are self-collected nasal and throat swabs. Self-collected nasal swabs and saliva samples can now be carried out in most European countries and the USA and then sent to the laboratories for analyses [25].

New testing strategies are required to promptly detect patients, lower waiting delays, and aid mass screening. Saliva (i.e. watery fluid secreted by the salivary glands in the mouth) is another alternative specimen. SARS-CoV-2 RNA has also been identified in faecal specimens, but faecal samples have not been recommended for routine analysis. Saliva specimen collection does not need trained staff and self-sampling may be performed. It is painless and easy to collect [26]. These samples (i.e. saliva and faecal specimens) are less invasive and reduce the risk of exposure to healthcare workers who collect specimens and the necessity for repeated substitution of personal protective equipment (PPE). These samples are required if there is a high suspicion of SARS-CoV-2 in patients with a negative RT-PCR result on respiratory samples. Swabs must be placed in suitable authorized liquid media and must be transported at ambient temperature to the laboratory for nucleic acid analyses [10].

Point-of-Care Nucleic Acid test or analysis

A point-of-care RT-PCR test is currently in application in most countries (Europe, Asia, etc.). There is no separate extraction step; the test takes a short time for a result to be available [about 45 minutes from the sample arrival time (i.e. short turn-around time, STAT)]. At present, simultaneous analysis of large numbers of specimens is not possible, because the reagent cartridges are of limited use. There are also available similar rapid analyses or assays which include multiplexed additional respiratory virus targets. These include, for example, QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN GmbH) and the Cobas SARS-CoV-2 and influenza A/B nucleic acid test (Roche Molecular Systems). Now in application in some point-of-care settings such as hospital emergency departments is the ID NOW COVID-19 assay (Abbott) [10] in some developed countries. This applies isothermal nucleic acid technology. The analytical sensitivity is low when compared to the other sample-to-answer application.

Laboratory Analysis of Nucleic acid

Commercially available in-house developed nucleic acid analyses are now available. The total time for the analysis is about six hours due to the extraction of the RNA from the specimens before amplification is required. The turnaround time is about 24 to 48 hours, because of the transport time and the need to batch specimens together. The ability to carry out a large volume of analyses at the same time is the advantage of these analyses. There are also available modern entirely incorporated sample-to-answer molecular diagnostic programs. These programs include Hologic's Panther Fusion and Aptima SARS-CoV-2 assays and Roche's Cobas SARS-CoV-2 test, which present throughput, and DiaSorin Molecular's Simplexa COVID-19 Direct kit, which in addition presents better turnaround times [10]. These modern equipment are now in use in Asia, Australia, the USA, Canada, and most countries in Europe.

Antibody Analysis

Testing capacity has increased globally in the past months; most of the COVID-19 cases have been limited to symptomatic patients and individuals having close contact with confirmed cases. Although, subclinical asymptomatic infections account for about 45 percent of infections and these are thought to be a very significant contributor to the transmission of SARS-CoV-2 [27], as SARS-CoV-2 has the possibility of continued viral shedding in asymptomatic cases. The serological identification of antibodies against the virus will offer or provide a major role in complementing molecular analyses to improve diagnostic accuracy, contact tracing, vaccine efficacy or evaluation testing, and seroprevalence surveillance at the local, state, and national level [28].

The RT-PCR analysis is also likely to give false negative results under certain conditions, thus omitting or leaving out some COVID-19 patients. These conditions include:

a. Bad timing for sample collection
b. Inadequate or inappropriate specimen for viral RNA extraction
c. Unsatisfactory specimen transportation
d. Deficient storage of the extracted RNA specimens
e. Reduced quality of RT-PCR analysis – this is due to unpredictable cycle threshold value and/or short of amplification signal for one or two targeted genetic materials (29).

Serological analyses, which identify antibodies specific for SARS-CoV-2 permit for a more correct assessment of the increasing prevalence of SARS-CoV-2 infection in a population compared to viral diagnostic analyses; as SARS-CoV-2 antibodies, in particular IgG, persist after viral clearance [30]. The serological analyses available at present for SARS-CoV-2 detect antibodies that are made against the viral protein spike [31-34]. Cases of SARS-CoV-2 infection have been confirmed to have an acute serological response [12, 35]. Thus, antibody responses, such as IgM, IgA, and IgG to SARS-CoV-2 in patient sera are detected by serological analyses. Serology is not recommended for the diagnosis of acute infection because of the late production of antibodies. There is an increase in seropositivity from day seven of the SARS-CoV-2 infection, with most cases seroconverting by day 14, although some cases can take up to 28 days. The antigen is planned to minimize cross-reaction against other human coronaviruses [11, 36].

Serology is suggested for:

a. Cases that had symptoms consistent with COVID-19 but had a negative RT-PCR result or have not been investigated
b. Cases with surprising or sudden positive or questionable or doubtful RT-PCR results
c. Supporting or backup with outbreak investigations and epidemiological surveys
d. Detecting or singling out convalescent cases for plasma donation

Presently, the duration of antibody response and correlation with protection and immunity to COVID-19 are both still unknown. It is being suggested that protection from reinfection may be more durable even though antibodies may decline over a few months. Serum must be taken from cases with well-matched clinical COVID-19 at least 14 days after the beginning of signs and symptoms. An early specimen that can be stored safely can be collected and applied as parallel analysis [2, 37].

Laboratory-based Antibody analyses

Serology for COVID-19 is carried out in the clinical laboratory by applying commercially available kits. The enzyme-linked immunosorbent assays (ELISAs) are applied by most clinical laboratories. Some reference laboratories apply:

a. Neutralization analyses,
b. Microsphere immunoassays, and
c. Immunofluorescence assays [10]

The commercially available assays have a sensitivity of 80 to 100 per-cent depending on the analysis with specificities of 98 to 100 percent [38].

Antibody Results Interpretation

Interpretation of SARS-CoV-2 serological results is generally recommended. Serological studies which can identify antibodies against SARS-CoV-2 infection have the capability to detect those who were asymptomatic [asymptomatic infection are individuals who have no clinical symptoms (e.g. fever, cough, or sore throat), yet give a positive result for the virus or serum antibody against SARS-CoV-2 [39] and were not detected by symptom reporting and hence provide a more complete picture of the prevalence and severity of COVID-19 disease (i.e. those who been infected with the virus).

A significant rise (e.g. at least fourfold) or increase in either neutralizing or IgG antibody levels or seroconversion is definitive laboratory support or indication of SARS-CoV-2 infection. The identification of IgG in the only sample from a case with well-matched clinical disease and with one or more clear or specific epidemiological conditions or factors for COVID-19 is indicative confirmation of SARS-CoV-2 infection. A follow-up specimen or sample must be requested in cases where only IgM, IgA, or both (i.e. IgM and IgA) are detected without IgG detection because this is not satisfactory or adequate evidence of SARS-CoV-2 infection [40, 41].

Antibody analyses by Lateral Flow Point-of-Care (POC)

SARS-CoV-2-specific IgM, IgG, and total antibody are now determined by a number of lateral flow point-of-care (POC) analyses in the developed countries including some African countries (e.g. South Africa). In order to give a quick or prompt turnaround time, blood specimens are collected from a vein or by finger prick. Due to the delayed antibody occurrence or response in SARS-CoV-2 infection, these analyses are not recommended for acute diagnosis. These analyses may also play a role in deciding or establishing immunity for return-to-work reasons or population surveillance [11, 41].

Antigen Analyses

The distinction between polymerase chain reaction- (PCR-) based analyses is that the PCR-based analyses detect the viral genome or RNA, while the immunoassays detect the presence of antiviral antibodies in the blood samples and these are patients which have successfully recovered from the infection (i.e. convalescent cases).

The antigen analyses are to detect the viral proteins. These are usually the nucleocapsid (N) protein and spike protein in the respiratory specimens and are applied for acute diagnosis of SARS-CoV-2 infection [10]. Usually, in the developed countries antigen analyses are suggested for the qualitative identification of SARS-CoV-2 antigens in official sample types taken from persons who are assumed to have COVID-19 by their healthcare provider in a definite number of days of the beginning of symptom [10].

Due to their reduced relative cost to RT-PCR and prompt turn-around time (TAT) and because it can also be scaled up to analyze large numbers of samples, there is a high interest in the application of antigen analyses. Their sensitivity to identify viruses compared to RT-PCR analyses are still of apprehension. The need for antigen analyses is reduced in most countries because of the widespread availability of RT-PCR analyses, usually with quick turn-around times (TAT). In certain situations, such as the high-risk settings may have a role for antigen analyses.

Personal Protective Equipment (PPE) during Specimen Collection

Adequate provision and distribution of personal protective equipment (PPE) are crucial for caring for patients during the pandemic. There should be adequate personal protective equipment (PPE) for frontline healthcare workers. The personal protective

equipment (PPE) that should be used when taking respiratory specimens in symptomatic cases suspected of having SARS-CoV-2 infection include:

a. Gloves
b. Surgical mask and face shields
c. Eye protection
d. Gowns – where and when necessary
e. Hand sanitizer

The necessity for a gown or apron must be based on a risk evaluation. When taking blood for serological analyses in asymptomatic cases of SARS-CoV-2 infection, there should also be a safety measure, or else standard precautions are satisfactory. It has been shown that personal protective equipment dampens the risk of transmission in a clinical setting [42-44]. In many countries shortages of personal protective equipment (PPE) for healthcare workers especially in some African countries have hindered or slowed down SARS-CoV-2 analyses [43]. In some African countries, there are also limited or nil laboratory testing facilities and insufficiently trained personnel. Laboratory analyses will also help to minimize the spread of the infection in African countries [45, 46].

CONCLUSION

COVID-19 continues to threaten the healthcare system and the world's economy. Harsh or Strict public health measures, for example, social distancing, contact tracing, testing, quarantines, and travel limitations are of principal significance to manage or check the spread of the virus.

For both the clinical monitoring and treatment of the cases with SARS-CoV-2 infection and to notify successive quarantining and isolation, prompt or quick detection by applying laboratory analyses is very important or crucial.

Thus, the correct selection of diagnostic analysis and type of specimen will increase or raise the chance of detecting positive cases, and thus reduce the needless anxiety and cost of analyses with irrelevant or little clinical application. Laboratory analyses of SARS-CoV-2 offer a valuable tool for COVID-19 management in detecting cases with critical conditions.

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