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Review Article

# Laboratory diagnosis of SARS-CoV-2 - A review of current methods

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ABSTRACT

At present the whole world is facing pandemic of the Coronavirus disease (COVID-19); caused by **severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)**. **This** disease **has** rapidly spreads across the world from its origin of Wuhan, **China and affected millions people worldwide and make them to remain in their homes**. The knowledge of available laboratory methods is essential for early and correct diagnosis of COVID-19 to identify **new cases as well as monitoring treatment of confirmed cases.** In this review we aim to provide the **updated** information about selection of specimens and **availability** of various diagnostic methods and their utility with current findings for the laboratory diagnosis of SARS-CoV-2 infection. This will guide the healthcare professionals and government organizations to make strategy for establishing diagnostic facilities for SARS-CoV-2 infections.



**Contents**



## Introduction

In December 2019 a major outbreak from Wuhan, was reported in the World Health Organization (WHO) country office of China. The COVID-19 is a highly infectious disease and after reporting the first case in china it has rapidly covered the entire world and affected the life of millions people. On 17th April there are a total 2,164,111 confirmed cases and 146,198 deaths of COVID-19 reported globally to the WHO [1]. Due to the occurrence of many cases worldwide; on January 30th, 2020 the World Health Organization (WHO) declared a Public Health Emergency of International Concern [2]. In India, at present the total number of active cases and deaths are reported 12,289 and 488 respectively by the government agencies [3]. This COVID-19 infection also affected badly the United States of America, Spain, Italy and other countries.

The occurrence of an epidemic of respiratory infections in Wuhan, China, the CDC country office China documented its etiology which is attributed by a novel virus belongs to coronavirus (CoV) family; which includes alpha, and gamma Coronoaviruses. The virus has wide range of host such as humans, other mammals, and birds. The infected one may does not exhibit symptoms and may remain asymptomatic and some may have severe symptoms in their respiratory and digestive organs [4,5]. Coronavirus is a RNA virus which contains approximately 27–32 kb of positive-

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sense single-stranded RNA [4,5]. Like other corona viruses the nCoV have at least six open reading frames (ORFs) and many other accessory genes. At the 5′ terminal two-thirds of the genome consists of two open reading frames (ORFs), ORF1 and ORF2. These ORF encodes two polyproteins, namely pp1a and pp1ab, and further cleaved into 11 and 16 proteins, respectively. At the 3′ terminal the various structural proteins are located such as nucleocapsid (N), membrane protein (M), an envelope protein (E), and spike (S). These viruses also contain some accessory protein, which help in virus replication. The S gene, help nCoV for host specificity and receptor binding, and some may also contain hemagglutinin- esterase (HE) protein in their virion [6].

The COVID-19 infected patients have clinical manifestation which includes the fever and cough as primary clinical presentations and others are shortness of breath and myalgia etc. Some patients may have serious complications such as acute respiratory distress syndrome (ARDS) and cytokine storm, which may leads to death [7]. **The collection of appropriate specimen is very crucial for detection of most of the infected cases of COVID-19. The nasopharyngeal swab usually collected, but in some instances we may miss the detection; therefore the lower respiratory tract specimens such as sputum, bronchoalveolar lavage (BAL) may be the alternate choice** [8].

In this pandemic of SARS-CoV-2 the reliable, early and accurate diagnosis is very crucial to provide timely medical help to the infected individual as well as it helps to the government agencies to prevent its spread to other individuals and saves people's lives. False negative test result may lead to spread of the epidemic in the community; **similarly the false positive result may lead unnecessary treatment and mental trauma to the patients. Therefore, there is an urgent need to have accurate, rapid, readily available and reliable diagnostic test for SARS-CoV-2 infection. Till date, various immunological and nucleic acid amplification diagnostic tests are developed and easily available. Various integrated point-of-care molecular devices are currently under development and some are available to provide accurate and fast diagnostic services for SARS-CoV-2 infections.**

In present crisis of COVID-19 an accessible and reliable testing is a need of hour. In this review we try to explore the selection of appropriate specimen; availability and utility of various diagnostic tools for the purpose of diagnosis of COVID-19 infected cases. This will help the **authorities** to **utiliz**e the most suitable diagnostic tool, **for tracking the virus and to take timely action to suppress the transmission** [9].

## Laboratory diagnosis of SARS-CoV-2 infection

### *Types of specimen*

As per Centre for Disease Control and Prevention (CDC) recommendations the upper respiratory specimens should be collected for RT-PCR based testing of COVID-19 and especially the nasopharyngeal specimen is a preferred choice [10]. The CDC also recommended that when the nasopharyngeal swab is not possible the other specimen such as; an oropharyngeal (OP) specimen, nasal mid-turbinate (NMT) swab, an anterior nares (nasal swab; NS) specimen and nasopharyngeal wash/aspirate or nasal aspirate (NA) specimen can be collected alternatively. All specimens should be placed in a tube containing viral transport medium and transported to the laboratory on time. Similarly for serological assay the blood sample can be collected. The healthcare professional should adhere to infection prevention and control guidelines of WHO and use the personal protective equipment such as, gown, gloves, eye protection and N95 mask while collecting the specimen [11]. If the shipping of specimen to the reference diagnostic laboratories required, **the sample must be transported in triple packaging system; the sample vial must be properly labeled and sealed and kept in outer covering of absorbent material (primary container) and then placed in secondary container**. **After that the secondary container should be placed with frozen gel packs in the thermocol box (outer container). All the processes; including packaging, labeling and shipping must be done as per the WHO guideline** [12].

Various studies conducted and have given evidence that the SARS-CoV-2 can invade digestives system and haematological system along with respiratory system [13]. Peng et al. from China; in their study they were collected different types of specimen for detection of SARS-CoV-2 RNA and found highest positivity rate in pharyngeal swab (78%), after that equally in blood and anal swab (22% each) and 11% in urine [13]. Another study published from United States, they found that stool, oropharyngeal swab and nasopaharyngeal swab were positive for SARS-CoV-2, while serum and urine were found negative. However this study is published as case report and included only one patient [14]. A study from china enrolled 41 patients; all are laboratory confirmed SARS-CoV2 infected. Among these only six blood specimens were found positive while all 41 respiratory specimens were found positive [15]. Another study from china found very interesting results that the virus can be detected in feces and blood along with respiratory specimens, however the percentage is not very promising [16].

In a recently published case report from Japan the investigators documented that when the clinical suspicion is high the negative PCR test on throat swabs is not sufficient to rule out the COVID-19 infection; but the bronchoalveolar lavage (BAL) of the lower respiratory tract specimen should be preferred for diagnosis [17]. Thus it is very important to conclude the final result of the highly suspected SARS-CoV-2 infected individuals; we should consider collecting various types of specimen. This will improve the detection rate and can reduce the false negative results. Similarly Peng et al. advocated the usefulness of testing of different specimen types for SARS-CoV-2 infection; to monitor disease changes and progression as well as for establishing a prognosis [13].

### *Virus culture*

The virus culture can be done by standard methodology as described by *Kim et al*; briefly the Vero cells which were cultured $1 \times$ Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum at 37 °C with 5% $CO_2$; used for the inoculation of nasopharyngeal and oropharyngeal samples [18]. And after 3 days of inoculation the specific cytopathic effects were observed. Later on they are also confirmed by using real time RT PCR. Researcher from wuhan, china have done virus isolation on human airway epithelial cells and Vero E6 and Huh-7 cell lines by inoculation of bronchoalveolar-lavage samples and the isolated virus was named 2019-nCoV [19]. Using the human airway epithelial cell cultures for virus isolation is skilled labor intensive task, however these were found very promising for analysis of respiratory pathogens of humans [20]. Recently an Indian study reported the First isolation of SARS-CoV-2 by using Vero CCL-81 cells [21]. The inoculated cells with nasopharyngeal and oropharyngeal samples, visualized for specific cytopathic effects for COVID-19 then these cells were fixed, dehydrated and cut into sections for transmission electron microscopy with standard methodology described by *Kim et al* [18]. *Kim et al* reported that they have observed Coronavirus-specific morphology and found the virus particle size ranged from 70 to 90 nm. They also found that the virus is observed in wide range of intracellular organelles especially in vesicles [18]. Viral culture of SARS-CoV-2 needs to be conducted in a bio-safety Level-3 facility. The cell culture is very useful for isolation and

characterization of viruses; but basically the cell culture for virus isolation is not recommended for diagnostic purposes.

*Immunological assay*

The immunological test measures the antibodies generated by host body's immune response against the virus infection or measures the proteins of COVID-19 virus present in the respiratory specimens. As virus enters in the human body it's elicit immune response to produce the antibody against the virus, detection of such antibody in infected person is very useful whether the person has symptoms or no symptom. Thus this type of tests provides valuable information about the person is exposed to this covid-19 or not. The only thing is that the antibody detection test is not for the identification of active cases of SARS-CoV-2 infections.

At the time of severe acute respiratory syndrome (SARS) epidemic, various reports documented that the detection of viral specific IgM and IgG are valid for serological diagnosis [22]. Xiang et al. [22] conducted a study in China and found that the serodiagnosis of COVID-19 based on IgM and IgG ELISA have great specificity for diagnosis of COVID-19. In their study they found that the sensitivity and specificity of detection of IgM were 77.3% & 100% and for IgG detection were 83.3.3% and 95.0% respectively; in the confirmed patients with COVID-19. Similarly in suspected COVID-19 cases the sensitivity and specificity were found for IgM 87.5% and 100% and for IgG were 70.8% & 96.6% respectively. Thus the detection of both IgG and IgM with higher specificity makes them reliable and could help us to establish the diagnosis of COVID-19 patients.

In general the lateral flow assay is used in the rapid point of care immunoassay. This assay could help us for rapid and on-site detection of COVID-19 especially in case of an emergency. These assays were developed to detect antigen of SARS CoV-2 virus or detecting IgG and IgM antibodies against the SARS CoV-2 virus infection [23]. Tang et al. emphasized that the detection of IgM and IgG antibodies by rapid lateral flow assay will play an important role in COVID-19 infections and help us to assess the burden of infections, find out asymptomatic patients etc [23]. COVID-19 IgM/IgG Rapid Test of BioMedomics is such of point of care device with 88.66% sensitivity available in the market [41]. Despite the rapidity and low cost of these immunoassays based on antigen detection for SARS-CoV-2; previous experience for influenza (Flu) viruses utilizing this type of assay has limitation of its uses. While utilizing this type of antigen detection test; one should keep in his mind that due to sampling variability and low viral load in the infected person, we may miss the case.

Serological assays are very rapidly developed and they measure the host immune response against the invading pathogens. Serological assay were used earlier in SARS and other corona virus outbreaks and played important role [24,25]. A study from china documented that by immunehistochemical analysis that we can detect the antigen in the lung tissue of the patients and the detection of IgM and IgG antiviral antibodies in the serum can provide additional evidences to confirm the COVID-19 cases [19]. A recent study from china analyzing the family cluster of SARS-CoV-2 infections revealed that the serology testing can assist in timely diagnosis while screening in close contacts [26]. Although WHO has recommended that the serology testing can be used where molecular testing is not available [27]. However the study from China emphasized the need of serology testing in this pandemic of COVID-19 due to high number of cases [26]. Immunological tests have limitation to use in early phase of infection because that time the immune response is still building. In case of COVID-19 infection we are in early phase of developing immunological diagnosis, some diagnostic test has been approved by Food and Drug administration of America, but still they are not recommended to use solely for the diagnosis of COVID-19 [28]. Similarly WHO also recommended that these points of care immunodiagnostic test used only in research setting not the purpose of clinical decision making until and unless the evidences also supported [29]. Even though certain limitations, in future these immunological tests may have critical role in the identification of individuals who have recovered from this COVID infection in the past. The test result may also help us in choosing the convalescent plasma, which can be used as treatment option for COVID-19 infected individuals [28].

*Nucleic acid testing assay*

In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions in nucleic acid testing assay. The real time reverse transcription–polymerase chain reaction (real time RT-PCR) is one of the best and accurate laboratory methods for detecting, tracking, and studying the coronavirus. Real time RT-PCR is a method by which we can detect the presence of specific target genetic material. Nowadays various fluorescent dyes are used as marker to detect the specific genetic target; earlier radioactive isotopes were used as marker. The most important aspect of using real-time RT-PCR assays is that the amplification and analysis will be carried out in a closed system; therefore the chances of false positive results will be minimized [30].

The Real time RT-PCR facilitates in analyzing the result in real time even though the process is still ongoing; which makes it more useful than conventional RT-PCR which provides the result at the end. The recent emergence of the coronavirus (COVID-19) has demonstrated the need of reliable and rapid detection, thus in present scenario the real time RT-PCR is the most widely used methods for the detection of coronavirus. The molecular testing is still a "gold standard" for relevant case diagnosis [26]. Most of the molecular diagnostics being developed are based on real-time RT-PCR assays for COVID-19 infection; loop-mediated isothermal amplification, clustered regularly interspaced short palindromic repeats and multiplex isothermal amplification followed by microarray detection are the some other methods which were developed and evaluated worldwide [23].

For conducting the PCR assay the number of molecular target have been identified within the RNA of Corona viruses; such as helicase (Hel), nucleocapsid (N), transmembrane (M), envelope (E) and envelope glycoproteins spike (S) [23]. Hemagglutinin-esterase (HE), open reading frames ORF1a and ORF1b and RNA-dependent RNA polymerase (RdRp), are some other genes that encode structural proteins can be utilized for the COVID-19 diagnosis [23]. In this real time –PCR assay the viral RNA is measured by the cycle threshold (Ct), which is defined as the number of cycles required for the fluorescent signal to cross the threshold and becomes detectable. The interpretation of result in real time-PCR is based on Ct values for specimen; a value less than 40 is clinically reported as PCR positive. In RT-PCR most of time results are 100% specific but false negative result may also occur; which may be due to sampling error or inappropriate timing of sampling [30].

The WHO recommended that the E gene assay followed by a confirmatory assay using the RdRp gene can be utilized for first line screening of COVID-19 cases [31]; and in the United States the CDC asked to use two nucleocapsid protein targets [N1 and N2] as molecular assay [32]. A study published from Hong Kong, China found that RdRp/Hel assay had the lowest limit of detection in vitro and have higher sensitivity and specificity among the three developed novel real-time RT-PCR assays targeting the RdRp/Hel, S, and N genes of SARS-CoV-2 [33].

It is advisable to use, at least two molecular targets to avoid the situation of a potential genetic drift of SARS-CoV-2 and the cross-reaction with other endemic coronaviruses as well However, the ideal design would include at least one conserved region and one specific region to mitigate against the effects of genetic drift,

**Table 1**
RT-PCR results of various studies for the diagnosis of SARs-CoV-2 infection.

| Author [reference] | Target | No of patients | Positive (%) | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Wong et al, 2019 [34] | RNA-dependent RNA polymerase (RdRp)/helicase (Hel) gene | 64 | 58 (91%) | 91% | NA |
| Yip et al, 2020 [35] | Non structural protein2 (nsp2) Real-Time RT-PCR Assay | 14 | 14 (100%) | 100% | NA |
| He et al, 2020 [36] | Open reading frame 1ab (ORF1ab) | 34 | 27 (79.4%) | 79 | 100 |
| Fang et al, 2020 [37] | Open reading frame 1ab (ORF1ab), nucleocapsid (N) gene and envelope (E) gene | 57 | 36 (63.15%) | 71% | NA |
| Liu et al, 2020 [38] | Open reading frame 1ab (ORF1ab) | 4880 | 2000 (40.98%) | NA | NA |
| | Nucleocapsid protein (NP) genes | 4880 | 1942 (39.8%) | NA | NA |

NA- Data Not available in article.

especially as the virus evolves within new populations [23]. Various studies conducted worldwide using molecular assay (RT-PCR Assay) for diagnosis of COVID-19 infections (Table 1). Most of the studies were used at least two target assay in combination for diagnosis of COVID-19 infections. In a study from Germany they have chosen envelope and RNA-dependent RNA polymerase [31]. In another study from Hong Kong, China the researcher first used the nucleocapsid for screening followed by confirmation by the open reading frame 1b [33]. **S**imilarly CDC conducted a study in the United States by selecting two loci in nucleocapsid gene and found the good performance for detection of COIVD-19 [32].

In view of the critical situations of COVID-19 infections worldwide, various companies attempted to develop commercial kits for detection of SARS-CoV-2 RNA by Real Time PCR. Various institutes of Indian Council of Medical Research, New Delhi (ICMR) till date (17/04/2020) evaluated the performance of 31 such commercial kits and among these 14 kits were found satisfactory [39].

Even though the RT-PCR is a tool to do definitive diagnosis of COVID-19, but the sensitivity is reported to be lower than Chest CT examinations. However; the chest CT alone does not differentiate between COVID-19 pneumonia to other viral Pneumonia [40]. Another molecular method that is Loop-mediated isothermal amplification (LAMP) reaction, could serve as an alternative method to the RT-qPCR to detect COVID-19. The LAMP is a nucleic acid amplification technique, which amplifies the DNA in isothermal condition with rapidity and high specificity. This method can be utilised for the diagnosis of COVId-19 without the need of specialized equipments and trained analysts. In near future the point-of-care device based on LAMP can be a potential diagnostic tool for the diagnosis of COVID-19 infected individuals [41]. Recently; Prof. Feng Zhang et al given a CRISPR-Cas13 (clustered regularly interspaced short palindromic repeats- and associated Cas proteins13) based SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) protocols, which is accurate and rapid method for Novel Coronavirus (COVID-19) [42]. Kim et al in their study reported that they have constructed the next generation sequencing (NGS) library by amplifying the full-length genes of the isolates using the synthesized cDNA and primers specific for SARS-CoV-2 [18].

Further development and inclusion of these new technologies for the diagnosis of COVID-19 can provide a better, accurate and rapid tool. These developments may also reduce the need of sophisticated equipments and specific training; this will help us to reach to a wide community for screening as well as for diagnosing them.

## Conclusion

In a present scenario the early diagnosis is very crucial to identify the infectious cases to prevent community transmission. At present various technologies are available to provide better diagnostic services to the community in this outbreak. There is need to collect the right specimen on right time for proper diagnosis. Due to high risk of infection, the challenges are to keep healthcare professional safe by having appropriate PPE kits. Real time RT-PCR assay remain the molecular test of choice for etiologic diagnosis of the COVID-19 cases; while antibody based immunological test are used as supplementary tools for screening the whole community and confirming with the molecular assay. Both real time RT-PCR and immunological assay help us to tackle this major outbreak of COVID-19, which impacted the life of people and global economy. The rapid test kits are in demand for providing rapid diagnostic services in an emergency situation as well at bed-side to the patients. In summary the proper utilization of available test in alone or in combination; we can detect the COVID-19 cases as soon as possible and saves lives of human being.

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