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# Laboratory testing for the diagnosis of COVID-19

Christopher K C Lai [a, *], Wilson Lam [b, c]

[a] Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China
[b] Chiron Medical Group, 2/F Podium Plaza, Hanoi Road, Hong Kong Special Administrative Region, China
[c] Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China

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ABSTRACT

Rapid and accurate laboratory diagnosis of active COVID-19 infection is one of the cornerstones of pandemic control. With the myriad of tests available in the market, the use of correct specimen type and laboratory-testing technique in the right clinical scenario could be challenging for non-specialists. In this mini-review, we will discuss the difference in diagnostic performance for different upper and lower respiratory tract specimens, and the role of blood and fecal specimens. We will analyze the performance characteristics of laboratory testing techniques of nucleic acid amplification tests, antigen detection tests, antibody detection tests, and point-of-care tests. Finally, the dynamics of viral replication and antibody production, and laboratory results interpretation in conjunction with clinical scenarios will be discussed.


## 1. Introduction

Since late 2019, coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread and infected millions worldwide. At the same time, laboratories decoded the virus's genome and worked on perfecting its diagnosis at a similarly relentless pace. With the first SARS-CoV-2 genome sequence published on 11 Jan 2020 (Genbank accession number MN908947), regional key laboratories swiftly came up with standardized laboratory diagnostic protocols, recommending the appropriate primer, probes, and thermocycling conditions to accurate diagnosis [1]. These standardized laboratory protocols enabled rapid testing for COVID-19 in established laboratories equipped with well-segregated pre-amplification, amplification, and post-amplification areas. They also formed the capstone for a myriad of commercially available "rapid-assays" that soon followed. These commercial assays varied in their detection technology, which range from detecting the SARS-CoV-2 antigens, antibody tests, a combo of antigen and antibody tests, to easy to use sample-to-answer nucleic acid amplification tests. They also varied in detection targets and thus performance characteristics.

Laboratory diagnostic tests are utilized in many scenarios: from asymptomatic population screening, targeted high-risk population screening, contact investigations, clinical diagnosis, disease severity monitoring, monitoring of infectivity, to retrospective population-wide screening. In our opinion, no single test can fulfill the requirement in every single scenario mentioned above. The purpose of the current review is to provide readers a summary of latest knowledge on the arsenal of COVID-19 tests available, enabling best use of the right diagnostic test in the required clinical context in the most cost-efficient way.

## 2. Specimen types

COVID-19 is a viral infection that mainly attacks the respiratory tract [2]. The choice of specimens depends on the testing scenario, clinical features, and stage of disease. Viral detection of specimens from the upper and lower respiratory tracts have been advocated as the main means of making the diagnosis of active clinical infection. Other specimen types, including serum and stool had also been suggested.

### 2.1. Upper respiratory specimens

Clinical samples are from the upper respiratory tract samples by using nasopharyngeal swabs, nasopharyngeal aspirates, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate swabs, or a combination. With the exception of anterior nasal swabs and mid-turbinate swabs that can be collected by patients [3], healthcare professionals are required to reliably collect upper tract specimens [34].

* Corresponding author. 1/F, Lui Che Woo Clinical Science Building, Prince of Wales Hospital, Shatin, NT, Hong Kong Special Administrative Region, China.
*E-mail address:* chris.kclai@cuhk.edu.hk (C.K.C. Lai).

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Upper respiratory specimens are recommended for making clinical diagnosis of active disease in symptomatic cases in early-stage infections or asymptomatic persons. Nasopharyngeal specimens, especially nasopharyngeal aspirates (NPA) were traditionally considered the specimen of choice for making diagnosis of respiratory viral infections, giving the highest yield in viral detection, such as in Influenza A [5,6]. With the potential risk of generating aerosols during the suction in collecting NPA [7], we do not recommended collecting NPA for clinical diagnosis in COVID-19.

Nasopharyngeal swabs (NPS) is a good alternative to NPA and give a more reliable result than oropharyngeal swabs [8–11]. To further improve diagnostic yield, combining nasopharyngeal and oropharyngeal swabs is now one of the most commonly used specimen type for the diagnosis of COVID-19 active infection [11–15].

Nasal swabs, mid-turbinate, and saliva specimens, being self-collected, has the advantage of not requiring collection by health-care professionals, especially when personal protective equipment is in short supply. Several studies have suggested that the relative sensitivity of self-collected saliva specimens compared with nasopharyngeal specimens is more than 85% [16–23]. However, the methods and instructions on collection varied between the studies and the optimal method remains uncertain, from gargling with saline solutions, spitting, collection of drools, or use of pipet of special sponges [18,19,22,24–27]. The use of deep-throat saliva, which is the self-collection of the posterior oropharyngeal secretion, was particularly studied in depth. The sensitivity is comparable to nasopharyngeal swab specimens [13,16,28]. As the result of heterogeneity of methods and study designs, the WHO do not currently recommend the use of saliva as the sole specimen type for clinical diagnosis of COVID-19 infection [4].

### 2.2. Lower respiratory specimens

Lower respiratory tract specimens have higher viral loads and are more likely to yield positive tests compared to upper respiratory tract specimens [9,29]. Lower respiratory specimens are recommended in the later course of COVID-19 disease, or in patients with strong clinical suspicion but upper respiratory specimens tested negative [4].

Sputum is the most easily collectable lower tract specimen if spontaneously produced. It has been shown to give a higher yield than upper respiratory specimens in COVID-19 [13]. Induced sputum is not recommended due to the risk of aerosol generated during the procedure, putting the healthcare workers at risk [30].

Endotracheal aspirates and brochoalveolar lavage can be collected from patients with severe disease and under closed-loop mechanical ventilation and strict adherence to infection control guidance in appropriate settings (e.g. well-ventilated negative pressure rooms).

### 2.3. Blood specimens

SARS-CoV-2 virus can be detected in plasma in rare occasions and can be regarded as marker of severe to critical disease [31,32], however, its role in aiding the clinical diagnosis is limited. In the setting of acute clinical disease, paired serum sample taking 2–4 weeks apart can be used to look for four-fold rise in antibody titres. However, serology cannot be used as a standalone diagnostic for acute SARS-CoV-2 infection [4]. The highest diagnostic value of blood in its use in determining the seroprevalence at population level.

### 2.4. Fecal specimens

SARS-CoV-2 virus is found to be present in a large proportion of COVID-19 patients, with higher chance in more severely ill patients [32,33], and in second week onwards [33]. Anal swabs were found to be independently associated with intensive care admission and can be considered as a warning indicator for severe disease [34]. Fecal specimens can also be considered in patients were respiratory tract specimens are negative despite strong clinical suspicion [4].

## 3. Laboratory testing technique

### 3.1. Nucleic acid amplification test (NAAT)

NAAT is the technology of choice to make a diagnosis of an active COVID-19 infection. Use of real time polymerase chain reaction (RT-PCR) assay to detect SARS-CoV-2 RNA from the upper respiratory tract is the preferred initial diagnostic test [35]. Other NAAT techniques, such as Loop-mediated isothermal amplification (LAMP)-based [36,37] and clustered regularly interspaced short palindromic repeats (CRISPR-based) assays has also been developed [38,39].

The NAAT assays targets the SARS-CoV-2 nucleocapsid (N), envelope (E), and spike (S) genes, and regions in the first open reading frame (*orf1a* and *orf1b*), and the RNA-dependent RNA polymerase (RdRp) gene [40–43]. When performing in-house assays, ideally two independent gene targets should be utilized as the performance of single target assays may be affected by viral mutations. When using commercially available assays ensure a means to keep track of possible performance variation due to cumulating genetic mutations [4].

Sample-to-answer molecular diagnostics platforms have the advantage over conventional molecular platforms that they do not require stringent laboratory setup. There are many such platforms granted emergency use authorization (EUA) by the US Food and Drug Administration (FDA) [44]. These systems required minimal hands-on time, excellent sensitivity and specificity, with rapid turnaround time [45,46]. The major downside of these platforms, in our opinion, are their cost per test with makes them financially unattractive in mass screening programs. These systems would still rely properly trained laboratory technicians and a laboratory quality assurance system should be in place to ensure quality results. There is no strong recommendation on whether these sample-to-answer platforms is preferable over standard molecular platforms in testing of symptomatic patients with a suspicion of COVID-19 [3]. Users are advised to consider according to the available resource, specimen batch size, and the required turn-around time.

The cycle threshold (Ct) of the RT-PCR assays refer to the number of cycles needed to amplify viral RNA to reach a detectable level. The Ct value is inversely related to the *relative* viral RNA level in a specimen. Ct values are not standardized to give quantitation of viral concentration and across RT-PCR platforms. Clinicians should take not of these limitations when attempt to use the Ct value as a guide for clinical management, especially when multiple diagnostic platforms or tests are used.

### 3.2. Antigen detection

Antigen detection tests detect the presence of SARS-CoV-2 viral proteins on respiratory samples. Most of the commercially available kits require samples taken from nasal cavity or nasopharynx, alternate samples like saliva have also been studied [47]. They utilizes immune-based technologies with different variations of detection like by lateral flow sandwich immunoassays, microfluidic

immunofluorescence assays, and chromatographic digital immmunoassays [44]. These test kits typically include all the required materials to perform the tests and are easy to perform and can be used as laboratory-based tests, or point-of-care tests (POCTs). They are also referred to as rapid diagnostics tests (RDT) as they can give results in 15–30 min. The viral nucleocapsid protein is the most commonly chosen target as it is present in high abundance in clinical samples.

They do not involve target protein amplification and are often less sensitive than NAATs. Only four kits had undergone stringent regulatory review by the US FDA [44]. Users of these antigen tests should be aware that the sensitivity varies significantly with different assays. The average sensitivity was 56% with a range from 0% to 95% [48–51], False positive occurs infrequently. The specificity are reported to be high (>97%) [47] and are most commonly due to cross-reaction with proteins in other human coronaviruses.

Antigen detection tests have the advantage of being simple to perform and can play a role particularly in the setting where accessibility to NAAT tests are limited, and test on patients where a high viral load is expected, for instance, in the early course of illness and within the first 5–7 days from symptom onset [52,53]. The WHO recommended the use of SARS-CoV-2 antigen tests in settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility, and within the first 5–7 days following the onset of symptoms [47].

### 3.3. Antibody detection

Non-quantitative antibody detection is particular useful in epidemiological surveys, where attack rate in a specific population can be determined. In contrast, semi-quantitative or quantitative assays that can quantify the level of antibody production can detect a change in antibody titre, although not considered to be the test of choice for acute infection, can play a role in diagnosing acute infection.

Antibody detection assays commonly target against two SARS-CoV-2 antigens: the nucleocapsid (N), or spike (S) protein. The detection technology also differs. For laboratory-based assays, enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays (CLIA) are commonly employed. While lateral flow immunoassays, colloidal gold or fluorescence-label techniques are most commonly utilized.

Their performance has been evaluated in systemic reviews and validation studies [54–57]. It is advisable for users to understand the performance characteristic of the tests they intend to pursue, and perform in-house verification tests before putting into service [4].

These tests also differ in the antibodies being measured: immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), total immunoglobulin, or in various combinations of above. In general, tests using IgG antibody or total immunoglobulin have higher accuracy than test detecting IgM antibody, IgA antibody, or IgM/IgG tests [58].

Cross-reactivity with other coronaviruses and other viral pathogens causing false positivity is a potential concern [59,60], especially when the pre-test probability of infection is expected to be very low [61]. To improve the diagnostic accuracy of serology assays, the US CDC suggests an alternative strategy of using a two-step testing algorithm using two different antibody assays, in which an initial positive test is confirmed by a second, independent antibody assay [62].

Serologic tests are less likely to be reactive in the first several days to weeks of infection, they have very limited utility for diagnosis in the acute setting [62,63]. If used, checking serology three to four weeks after the onset of symptoms optimizes the accuracy of testing [58].

Virus neutralization assays required highly specialized skills, equipment, and requires BSL-3 facilities. They are considered the gold standard but are not for routine clinical diagnostic services. The role of detecting IgA in routine diagnostic tests has yet been established.

### 3.4. Point-of-care tests

Point-of-care tests are easy to use devices that can be used outside the laboratory settings. Most of the marketed POCT utilizes the detection of SARS-CoV-2 antigen or host antibody. As of April 2020, WHO does not recommend the use of antigen-detecting rapid diagnostic tests or antibody-detecting rapid diagnostics tests for patient care [64]. Users of these POCTs should also bear in mind that these tests may have lower sensitivity than laboratory based tests [65].

## 4. Interpretation

### 4.1. Dynamics of viral replication and antibody production

It was observed that the viral load detected in respiratory specimens began 5–6 days before and peaked around the time of symptom onset and subsequently declined in the following week [24,66,67]. That is important as with good sampling technique the false negative rate is expected to be low even in very early stage of disease.

Antibodies production take several days to weeks to develop [68–70]. In a systematic review of 38 studies that evaluated the sensitivity of antibody testing by time since symptom onset, IgM was detected in 23% by one week, in 58% by two weeks, and in 75% by three weeks; the corresponding detection rates for IgG were 30, 66, and 88% [54]. Other studies have suggested that the rate of positive IgG approaches 100% by 16–20 days [58,71,72]. Antibody levels are expected to reach the peak level at around three to four weeks from symptom onset. Therefore paired sera collected at least 14 days apart can be used for diagnosis of recent infection using semi-quantitative or quantitative assays. Antibody production has been observed to be faster and with a higher level in those with severe disease as compared to those with mild or asymptomatic infections [54,67,73,74]. The duration of detectable antibodies is still uncertain [75,76]. Some study found that IgG levels declined significantly early and at eight weeks following infection, 40% of asymptomatic patients and 13% of symptomatic patients did not have detectable IgG [75]. In contrast, in another study of 1107 confirmed cases, total antibody tests were reactive in 90%, with titers increasing over the first two months after diagnosis and remaining steady for another two months [77].

### 4.2. Test performance and result interpretation

In an analysis of seven studies (including two unpublished reports) that evaluated RT-PCR performance by time since symptom onset or exposure, the estimated rates of false-negative results were 100% on the day of exposure, 38% on day 5 (estimated as the first day of symptoms), 20% at day 8, and 66% at day 21 [23].

NAAT to detect SARS-CoV-2 RNA from the upper respiratory tract is considered the preferred initial diagnostic test for COVID-19 in the USA [35]. Antigen testing may be the initial test used in settings where NAAT test is not readily available, but as mentioned above, the sensitivity of antigen tests is lower than that of NAATs, and negative antigen tests should be confirmed with an NAAT test if clinically suspected.

A positive NAAT generally confirms the diagnosis of COVID-19.

The continual detection of SARS-CoV-2 RNA for weeks after symptom onset may signify the detection of non-viable viral fragments and does not equate infectiousness [67].

A false-negative NAAT result can occur, retesting can be considered 24 to 48 after initial test, and lower respiratory tract specimens can be saved especially when there is evidence of lower respiratory tract infection. We advise to always interpret laboratory results with caution. Always resample and retest whenever the test result is incompatible with clinical presentation, or weak positive NAAT results with high Ct values or unusual melting curve. Retesting can be performed by an alternate NAAT test, or seek reference laboratory confirmation when conflicting results are encountered such as contradicting results from two different assays.

A negative test result do not necessary rule out infection [8,78–82], False negative results can occur in preanalytical steps (poor specimen collection, inappropriate sampling), analytical steps (PCR inhibition, target mutation), and postanalytical steps (transcription error).

## 5. Conclusions

Diagnosis of COVID-19 is of paramount importance in the clinical management of SARS-CoV-2 infection and in curbing the ongoing pandemic. The current gold standard for detecting active COVID-19 remains viral detection by NAAT in respiratory specimens, which may not be readily available to less resourceful areas or limited in supply in many places. Users of antigen, antibody tests and POCT should be aware of their limitations. New diagnostic technologies with minimal hardware requirement, rapid turnaround time, and good performance is eagerly awaited to help control the pandemic or progression from mitigation to the new norm before availability and rollout of effective vaccines.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.10.069.

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