Review Article

Laboratory Testing for Diagnosis of COVID-19

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Abstract

SARS-CoV-2 variants have been emerging rapidly worldwide. To prevent and control the spread of SARS-CoV-2 variants, intensive study of virology, epidemiology, clinical characteristics, and genetic characterization of the variant strains is in progress. At present, vaccines against these multiple variants are being developed based on newly available genomic data from many recent studies. Surveillance of new variant strains is critically important to actively support public health control measures. It is necessary to detect the variants by viral diagnostic tests including Nucleic Acid Amplification Test (NAAT) and antigen tests. Different laboratory tests for identification of COVID-19 infection are performed depending on purposes such as risk of infection, active case finding and community spread control. To perform appropriate tests for surveillance, laboratory diagnostic tests are reviewed for accurate interpretation and effectiveness of surveillance.

Keywords: SARS-CoV-2 variants, COVID-19 infection, Laboratory diagnostic tests

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Introduction

COVID-19 infection caused by SARS-CoV-2 has become a global health emergency. Variants of SARS-CoV-2 have been emerging and spreading worldwide. The current variants, i.e., Alpha, Beta, Gamma, and Delta, are classified by the US government SARS-CoV-2 Interagency Group (SIG) of the Center for Disease Control and Prevention (CDC) as Variants of Concern because they are highly transmissible and severe and they significantly affect diagnosis and treatment or vaccination.[1]

The first significant evidence of SARS-CoV-2 transmission from human to human was reported in 2019.[2, 3] More than 370,000 cases were reported almost every day in late June 2021. The cumulative number of cases reported globally now exceeds 180 million and the number of deaths is almost 4 million. Globally, cases of the Alpha, Beta, Gamma, and Delta variants have been reported in 172, 120, 72, and 96 countries, respectively. Delta is continually emerging in more new countries with almost 3 million new cases and almost 56,000 new deaths in early July, 2021.[4, 5]

Clinical manifestations of viral respiratory infections vary from asymptomatic or flu-like symptoms to severe symptoms.[6] COVID-19 infected persons may have mild, moderate, or severe symptoms. Common symptoms include fever, chills, body ache, fatigue, headache, sore throat, rhinorrhea, dyspnea, chest pain, and cough with sputum. Other symptoms such as loss of smell and taste, nausea, vomiting, and diarrhea are less frequently encountered.[7] More severe symptoms include high fever, severe cough, and pneumonia. Neurological, gastrointestinal and respiratory symptoms may occur individually or combination of these symptoms.[8] Clinical manifestations of COVID-19 with more systematic symptoms and more severe radiological abnormalities have been found in older patients.[3] In most people, symptoms appear 7 - 13 days following infection. The current Delta variant produces slightly different symptoms than other variants and it is more contagious and more hazardous in vulnerable people, including vaccinated elderly people, and unvaccinated young children. It spreads more rapidly than other strains, including the Alpha and Wuhan strains of SARS-CoV-2.[9, 10] The differences between COVID-19 and other respiratory illnesses cannot be distinguished based on symptoms alone. Moreover, both influenza and COVID-19 can cause illness at the same time. Therefore, diagnostic tests are necessary for confirmation.[11, 12]

An early study on the pandemic revealed a high prevalence of asymptomatic infections (81%).[13] A meta-analysis revealed that the asymptomatic proportion ranged from 4% to 41% while meta-analysis with fixed effects showed a rate of the asymptomatic presentation during a follow-up period of at least 7 days was 17%. Presentation of asymptomatic COVID-19 infection was reported among healthy persons and younger age groups, mainly in children. Silent transmission of the virus from the asymptomatic people may spread the virus, but less than transmission from the infected people with symptoms.[3, 14, 15] However, a previous study revealed asymptomatic patients with COVID-19 infection presented abnormalities on chest CT.[2]

SAR-CoV-2 detection is crucially important for surveillance of strains to reduce COVID-19 spread. This review will focus on microbiology aspects of collecting specimens and methods for detection of SAR-CoV-2 to provide updated appropriate tests and guidance for investigation of COVID-19 infection.

Results

Patients with productive coughs are advised to have initial diagnostic testing for COVID-19. Specimen collection including from upper respiratory and lower respiratory tracts should be performed as soon as possible regardless of the time of symptom onset.[16] For current infection, viral tests are used. Antibody tests (serological tests) are used for past infection. The appropriate selection of test type is important for accurate diagnosis and control of COVID-19 spread.

Assays for SAR-CoV-2 using nucleic acid amplification tests (NAATs), such as real-time reverse-transcription polymerase chain reaction (RT-PCR) test have been developed to detect and identify RNA from upper or lower respiratory tract specimens of patients who are in acute phase of infection. The RT-PCR test is a nucleic-acid-based diagnostic tool with high sensitivity and specificity. In addition, SAR-CoV-2 antigen tests are used to detect SAR-CoV-2 proteins produced by the virus

in respiratory secretions. Both laboratory-based tests and near-patient tests are available. Antigen tests are reliable, inexpensive and fast, also known as rapid diagnostic tests, or Ag-RDTs. However, selection of SAR-CoV-2 Ag-RDTs is recommended to be at least ≥ 80% sensitivity and ≥ 97% specificity compared to a NAAT reference assay.[17]

Viral tests for current infection are recommended for: (1) people with COVID-19 symptoms, including fully vaccinated people, (2) asymptomatic people who have close contact with an infected person, (3) asymptomatic people who have received a positive test result within the past 10 days, and (4) asymptomatic people who participate in crowded indoor activities. The test is not necessary for fully vaccinated people who are not exposed to crowded settings such as schools, nursing homes, places of worship, correctional facilities, hospitals, social settings or workplace settings. Furthermore, persons who have tested positive within the past 3 months and recovered with no new symptoms do not need to test.[18, 19]

Collection of specimen

Specimens used for COVID-19 testing are collected from swabs of upper respiratory tracts (nasopharyngeal (NP), oropharyngeal (OP) or anterior nares) and lower respiratory tracts (sputum).

Nasopharyngeal specimens are collected by using synthetic fiber swabs with thin plastic or wire shafts. Oropharyngeal (throat) swab is acceptable but not recommended. Other types of swabs such as calcium alginate swabs or swabs with wooden shafts should be avoided as they may interfere with the tests. In case of saliva collection, 1 - 5 mL of saliva is taken into a sterile, leak-proof, screw cap container and the instructions from manufacturer are followed.[20]

To collect sputum, a 2 - 3 mL specimen from the lower respiratory tract is collected in a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Sputum, from a deep cough after rinsing the mouth with water, is collected directly into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

Collections of the lower respiratory tract which involve broncho alveolar lavage, tracheal aspirate, pleural fluid, and lung biopsy are used only for patients who have severe symptoms. Highly skilled physicians and specialized tools are necessary for collection in these cases.

1. Collection of Nasopharyngeal (NP) specimen

While patient's neck is in an extended position, a sterile nasopharyngeal swab is gently inserted into the nostril through the posterior nasopharyngeal wall. The swab is gently rotated to absorb secretions and then placed in transport media.[16]

2. Collection of Oropharyngeal (OP) specimen

A swab is inserted and rubbed over both tonsillar pillars and posterior oropharynx, including inflamed areas. Touching the tongue, teeth, and gums should be avoided.[16]

3. Collection of Anterior Nares (Nasal) specimen

A flocked or spun polyester swab is inserted inside the nostril (naris) and rotated to collect nasal membrane. The swab is placed for 10 to 15 seconds. Both nostrils are collected with the same swab.[16]

4. Collection of sputum

Deep cough sputum is collected into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container after rinsing mouth with water. The specimen is refrigerated at 2 - 8°C during transportation.[16]

Specimen handling

Specimen is immediately placed in transport media, aseptically. Patient's name, ID number, specimen type, and date collected are labelled. Caps are tightened to prevent leaking and specimens are placed in transport bags. Specimens are stored at 2 - 8°C for up to 72 hours after collection. If delay is expected in testing or shipping, specimens should be stored at -70°C or below.[16, 20]

Handling specimen and biosafety guidance in laboratory

Clinical specimens of suspected or confirmed positive for SAR-CoV-2 should be handled with precautions to prevent possible SARS-CoV-2 infection. Standard precautions include hand hygiene and the use of specific personal protective

equipment (PPE) including laboratory coats or gowns, gloves, eye protection, or a disposable mask and face shield, to protect skin and mucous membranes of the eyes, nose, and mouth.

Laboratory-acquired infection is a critical concern as infectious agents in specimens are potentially transmitted to laboratory personnel during laboratory processes. The risks of unexpected incidents should be effectively minimized by performing a risk assessment, providing a secure work setting and standard operating procedure for biosafety in a laboratory. Guidelines of biosafety for handling SARS-CoV-2 specimens in laboratories are provided by CDC.[21] Shipping of suspected and confirmed SARS-CoV-2 positive clinical specimens, cultures, or isolates should be packed as UN 3373 Biological Substance, Category B. BSL-3 practices in a biosafety level 3 (BSL-3) laboratory should be employed for cultures of SARS-CoV-2. In addition, appropriate communication among public health staff, including physicians, laboratory personnel, and specimen handlers, is needed in order to reduce potential risk of SARS-CoV-2 infection from handling any possibly infectious specimen.[22]

Testing for COVID-19

Initial viral diagnosis tests for current SARS-CoV-2 infections are Nucleic Acid Amplification Test (NAAT) and antigen tests. NAAT was recommended by Centers for Disease Control and Prevention (CDC) to identify the RNA sequences of the SARS-CoV-2 virus in specimens from upper respiratory tracts. Antibody tests are used, but not for diagnosis of current infection. Antibodies may develop 6 - 14 days after infection.

The requirements of specimens for tests vary according to each manufacturer's instructions. Nasal swab or nasopharyngeal swab is required for real time PCR and RADT. Saliva is used for Fast PCR. Nasal swab or Saliva can be used for LAMP. Meta-analysis revealed that specimens for SARS-CoV-2 detection can be either saliva or nasopharyngeal swab in both symptomatic and asymptomatic carriers. Saliva is used for surveillance, screening and diagnosis as collection can be self-administered and non-invasive.[23, 24]

COVID-19 diagnosis test is considered for the following conditions: (1) when symptoms of COVID-19 are present regardless of full vaccination or previous infection, (2) close contact with a confirmed COVID-19 infected person for a period of 15 minutes or more within 24 hours, (3) having activities in a crowded settings with poor ventilation, and (4) a test is requested by healthcare providers or an organization. COVID-19 testing is not recommended for a person who is fully vaccinated with no symptoms, despite being exposed to a positive COVID-19 person. In case of having symptoms or being potentially exposed to an infected person, it is suggested to be isolated from others pending test results and to follow the instruction of public health personnel.[19]

Surveillance for COVID-19 variants is routinely carried out by public health agencies. Once a variant is identified, enhanced testing should be performed for people who have any symptoms of COVID-19. Quarantine is not necessary unless the COVID-19 test is positive. Subsequently, genomic sequencing is carried out to monitor and reduce the spread of COVID-19 and to provide a better understanding of new variants for effective prevention and control measures.[25]

Testing for COVID-19 infection is performed after five or more days of quarantine.[26] If a test result is negative after day 7 or no symptoms have appeared by day 10 without testing, it is appropriate to stop quarantine. On the contrary, after exposure, the symptoms should be observed for 14 days and "the new normal" continued to slow down and prevent the spread of COVID-19. The new normal includes social and physical distancing at least 6 feet from others, wearing a mask, washing hands, staying away from crowds and poorly ventilated areas, covering coughs and sneezes, and cleaning and disinfecting frequently touched surfaces. Moreover, less than 14 days quarantine is recommended to reduce burden against a small risk of COVID-19 spread. However, new information is needed for updating recommendations.[27] Guidelines to help public health personnel decide when and how to test for suspected reinfection were developed by CDC and the guidance will be updated from ongoing COVID-19 studies with more detailed understanding of COVID-19 reinfection as rare cases of reinfection were reported.[28] Currently, COVID-19 tests have been developed for high sensitivity and specificity as described below.

1. PCR (polymerase chain reaction) tests are used to detect genetic material of SARS-Cov-2 in specimen. The tests include real-time PCR as a gold standard method and fast PCR tests which are the most sensitive and accurate tests available. This method is very useful to detect new variants due to mutations by designing multiple targets of the same virus. In case of discordant COVID-19 results with false negative RT-PCR testing, follow-up testing is suggested.[29]

2. RADTs (Rapid antigen detection tests) or point of care (POC) antigen tests are tests for specific viral antigens (proteins) on the surface of the virus. This method gives a high false-negative rate. False positive from cross reaction may also occur. Some countries have not adopted this rapid test.[30] As reported previously, the WHO (Nov 2020) stated that a rapid test which is ≥ 90% sensitivity and 99% specificity is used only if RT-PCR is not available. Currently, certified RADTs with high sensitivity are used to screen individuals for SARS-CoV-2 at international airports. In Thailand, RADTs is certified by Food and Drug Administration, Ministry of Public Health before using in practice. Guideline for RADTs is used in case of many patients to be tested and confirmed by RT-PCR. Self-test kits is also available for COVID-19 test. Specimen collection can be nasal swab or saliva.[31]

Appropriate methods for viral investigation are listed below.[32]

1. To confirm Patient Under Investigation: PUI, standard method, RT-PCR is performed by testing with specimen from posterior nasopharyngeal and throat or only nasopharyngeal.

2. To find active cases, for endemic in restricted region with few patients, apply the same guideline as mentioned in PUI. In case of endemic covering a wide region with high prevalence, use saliva for testing and report individually or loop-mediated isothermal amplification (LAMP), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) to triage patients appropriately and rapidly manage results. If positive result, confirm by standard method and conduct surveillance of the negative patients. For patients who are at high risk but negative result for COVID-19, RT-PCR should be used. Lastly, for epidemiological survey or investigation in high-risk area with low prevalence of COVID-19 infection, pool samples from nasopharyngeal/throat of 4 cases or from saliva of 5 cases for 1 pool to be tested.

Discussion

COVID-19 infections have increased globally. Pre-symptomatic patients can develop symptoms at any time within 6 days after the first detection of positive COVID-19 by RT-PCR test. However, most asymptomatic infected persons remain asymptomatic throughout the infection period.[33, 34] More new variants of SAR-CoV-2 strains are emerging worldwide. Effective strategies of controlling the spread are urgently needed to prevent community transmission. Because of asymptomatic infections and because the virus has been detected in stool, mitigation measures are advocated, including social distancing, wearing masks, regularly washing hands, and routinely sanitizing bathrooms. Quarantine for persons who have COVID-19 symptoms or persons who are not vaccinated and exposed to infected persons is critically important to prevent them from spreading the disease before they know that they are sick or infected without symptoms.[35]

The high prevalence of asymptomatic COVID-19-positive patients has been causing undetected transmission.[36] These asymptomatic patients should be identified rapidly for epidemic control. However, tracking and evaluating transmission from asymptomatic patients is complex. In addition, it is likely that pre-symptomatic and minor symptomatic cases unknowingly contribute to community and cluster infections more than asymptomatic patients. Accurate evaluation of prevalence requires repeated and widespread use of RT-PCR and antibody testing for developing effective public health strategies.[37] Confirmation test and follow-up testing should be performed after screening with negative result and discordant COVID-19 results.

Antigen tests taken either too early or too late in the course of infection may not detect the virus. Antigen tests vary in sensitivity. COVID-19 diagnostic tests with the sensitivity ≥ 80% and the specificity ≥ 97% is considered acceptable. Antigen tests are most detectable within the first week of symptom onset as the viral load is greater. The highest concentration of virus is found in nasal secretion. In asymptomatic cases, the sensitivity of the test ranged from 40% - 74%. Some people test negative when they actually do have the virus.[38]

The sensitivity of an initial reverse transcriptase PCR test was 87.8% from 16 studies and antibody tests ranged from 18.4% to 96.1% and the specificity 88.9% to 100%. Different type of samples for tests is possibly an influential factor for the accuracy of the tests.[37] A high false-negative rate of RT-PCR diagnosis due to inappropriate specimen collection and viral colonization was previously reported.[39]

For LAMP method, the test is less sensitive but it is simple and easy technique for application at the point of care as previous reports.[40, 41] Antibody test or serological assays is used for screening as the tests have high throughput capacity and less stringent specimen requirements than RNA-based assays. However, false positives using COVID-19 testing with high sensitivity and the specificity are currently reported. Further confirmation test should be performed in case of people who have no indicative histories.[42] To be concerned of the uncertainty of immunity level, people testing positive for antibodies should still maintain protective measures to prevent further spread.

Genetic sequencing is an important method for identifying new emerging variants. Knowing the genetic characteristics of SAR-CoV-2 variant strains improves the understanding of COVID-19 severity, diagnostic tests, treatment, and vaccine effectiveness.[43] Close surveillance testing is the key strategy to stop the spread. More infection generates more opportunity of mutation into contagious variants such as the Delta strain. Surveillance testing of asymptomatic people throughout a community can help to control the spread of COVID-19 infection.

In conclusion, it is critically important to combine quarantine, surveillance of suspected patients, and rapid viral diagnostic testing to control rapid spread of COVID-19 infection. Further investigations of the mechanisms of COVID-19 acquisition and of SAR-CoV-2 transmission are needed to improve diagnostic testing and treatment of infection.

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Conflict of interest

Potential conflict of interest. All authors report no conflict of interest relevant to this article.

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