Review Article

Laboratory Diagnostic Testing for COVID-19

Nuntra Suwantarat[1], Sidayu Suriya[2]

Abstract

Laboratory tests play an important role for Corona Virus Disease 2019 (COVID-19) diagnosis. The diagnostic key point is an appropriate testing during the course of disease. In the early course of COVID-19 infection, nucleic acid amplification tests (NAATs) especially real-time reverse-transcription polymerase chain reaction (RT-PCR) of the respiratory specimen is the gold standard test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection due to the highest sensitivity and specificity during the first 2 weeks after the exposure. A rapid antigen test for SARS-CoV-2 has less complexity of the testing procedure and the faster TAT. However, a rapid antigen test has less sensitivity compared to NAATs. Serological tests for COVID-19 are recommended for assisting the diagnosis in some indications and focused on epidemiological study. Viral isolation and genomic sequencing are not routinely used for COVID-19 diagnoses, but they are important for the study of viral mutation and outbreak investigation.

Keywords: Laboratory test, COVID-19, SARS-CoV-2, Molecular test, Serology, WGS

Received: 6 July 2021 **Revised: 27 July 2021** **Accepted: 27 July 2021**

[1] Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 12120, Thailand
[2] Department of Medicine, Faculty of Medicine, Naresuan University, Phitsanulok 65000, Thailand
Corresponding author: Nuntra Suwantarat, Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 10120, Thailand
Tel. +66 2564 4440-9 ext. 4496, +669 6363 9541 Email: nsuwantarat@gmail.com

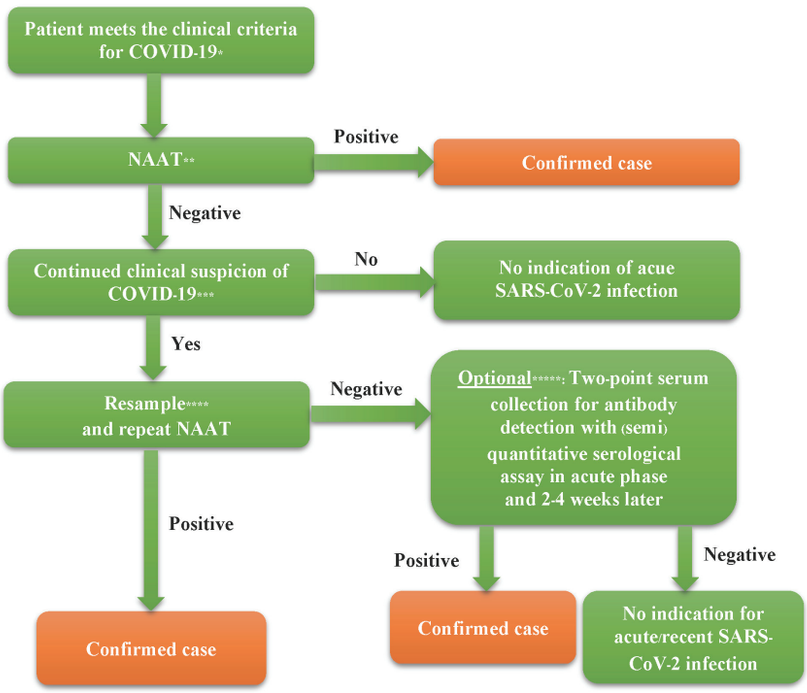
Introduction

COVID-19 is an emerging infectious disease since the first report of a novel coronavirus from patients with pneumonia in December 2019 in China. The world was recognized as a cluster of 3 patients with severe pneumonia of unknown cause that was linked to a seafood market in Wuhan, China. Initially, the polymerase chain reaction (PCR) for 18 viruses and 4 bacteria were not detected any pathogens from lower respiratory tract specimens from these patients. Thus, high throughput genomic sequencing was used to discover a novel coronavirus (2019-nCoV). The schematic of and phylogenetic analysis of 2019-nCoV was performed and confirmed the 2019-nCoV as a single-stranded ribonucleic acid (RNA) virus within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family Coronaviridae and the Orthocoronavirinae subfamily which includes severe acute respiratory distress syndrome coronavirus (SARS-CoV) and Middle East respiratory distress syndrome coronavirus (MERS-CoV). Viral isolation from the patients' lower respiratory tract samples in the human airway epithelial cells culture showed cytopathic effect (viral growth) and visualized of 2019-nCoV by using transmission electron microscopy.[1] Finally, the World Health Organization (WHO) officially announced for the coronavirus disease (COVID-19) in the International Classification of Diseases (ICD) and the International Committee on Taxonomy of Viruses (ICTV) named 2019-nCoV as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on 11 February 2020.[2, 3] In this review, we will focus on laboratory testing for SARS-CoV-2 especially on the routine laboratory test for COVID-19 diagnosis and practical key points for applications such as nucleic acid amplification tests (NAATs), antigen and serological tests. Overall, the consideration of the testing is based on 1) course of disease (duration of exposure, phase of disease, peak viral loads) and 2) laboratory identification technique (sensitivity and specificity of the test, complexity of the test, limitation, type of specimens, turn-around time (TAT), cost, and feasibility). Viral culture is not routinely used for COVID-19 diagnosis. However, it is important for understanding viral growth and possible related to the duration of disease transmission. Moreover, Genome sequencing and typing especially with next-generation sequencing and bioinformatics are helpful for outbreak investigation, identification of pathogens including variant types and mutation, and understanding of disease transmission.[1, 4-6]

Laboratory Diagnostic Testing for COVID-19

1. Nucleic acid amplification tests (NAATs)

Molecular tests, especially NAATs are the gold standard for COVID-19 diagnosis especially in symptomatic patients with early clinical disease and asymptomatic patients with history of high-risk contact based on the World Health Organization (WHO), U.S. Centers for Disease Control and Prevention (CDC) and Infectious Disease Society of America (IDSA)'s recommendation on diagnostic testing for SARS-CoV-2 (Figure 1).[7-9] SARS-CoV-2 is an enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome.[3] The genome encodes for non-structural proteins and four structural proteins; spike (S), envelope (E), membrane (M), nucleocapsid (N), and accessory proteins.[10-12] There are several types of NAATs. The detection of unique viral sequences by real-time reverse-transcription polymerase chain reaction (RT-PCR) is the standard confirmation of acute SARS-CoV-2 infections. The RT-PCR assays' targets include regions on SARS-CoV-2 genome such as the E, RdRP, ORF1ab (ORF1a, ORF1b), N and S genes (Figure 2).[7, 8, 10-12] The RT-PCR will amplify and detect the SARS-CoV-2 genome. In Thailand, the hospital or institutional laboratories must be certified and accredited for SARS-CoV-2 detection by the Department of Medical Science, Ministry of Public Health (MOPH) prior to operate as a routine laboratory service.[13]



*	Clinical management of COVID-19 (interim Guidance), World Health Organization.
**	If antigen detection would be incorporated into the testing algorithm how this needs to be done depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. For more information see section below on "Rapid diagnostic tests based on antigen detection" and the specific guidance interim guidance on antigen-detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays.
***	Continued clinical suspicion can, for example, be the absence of another obvious etiology, the presence of an epidemiological link, or suggestive clinical finding (e.g.typical radiological signs).
****	The selection of specimen type will depend on the clinical presentation, see section "specimens to be collected". Increasing the number of samples tested will also increase the sensitivit of testing for COVID-19. More than two samples might be needed on some occasions to detect SARS-CoV-2.
*****	For interpretation of serology, see section "Implementation and interpretation of antibody testing in the clinical laboratory". Serology cannot be used as a standalone diagnostic for acute SARS-CoV-2 infections and clinical management.

Figure 1 COVID-19 diagnosis guideline.

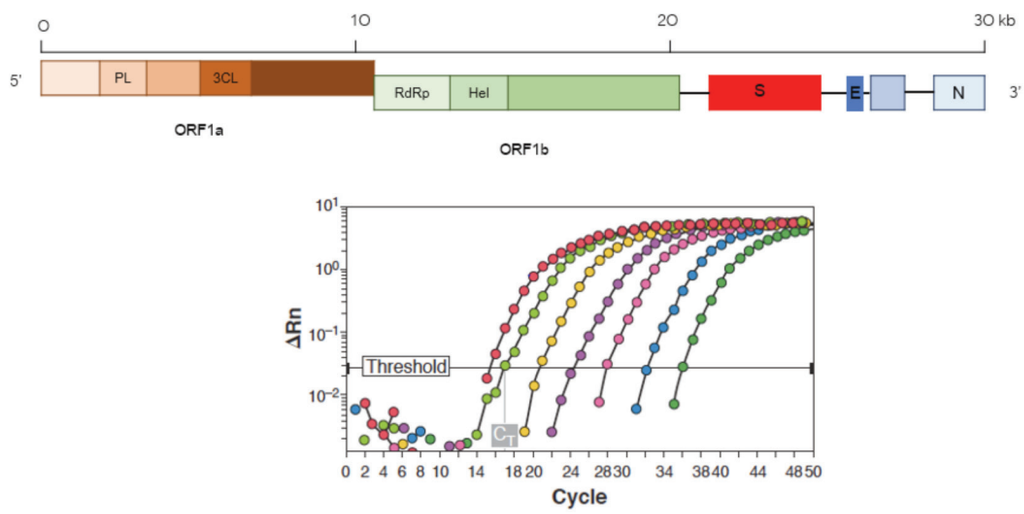


Figure 2 Diagram of SARS-CoV-2 genome and example of RT-PCR cycle threshold curve.

Real-time reverse transcription polymerase chain reaction (RT-PCR)

The detection of SARS-CoV-2 by RT-PCR from nasopharyngeal (NP) swabs are currently the reference tests for COVID-19 diagnosis.[2, 8, 9] It is due to a high sensitivity of 0.98 (95%CI: 0.95 to 0.99) and high specificity of 0.97 (95%CI: 0.92 to 0.99).[14-25] In the patients with COVID-19, the incubation period is usually 5 - 6 days, with a range of between 1 - 14 days following exposure.[26-30] The virus may be detectable in the upper respiratory tract (URT) specimens 1 - 3 days before the onset of symptoms. The peak of viral RNA in the URT is highest around the time of symptom onset and gradually declines.[31-37] The presence of viral RNA in the lower respiratory tract (LRT), and subsequence in the feces, increases during the second week of disease. The range of detectable viral RNA varies from several days to months.[33]

Traditional RT-PCR is a standard laboratory technique of molecular biology based on the nucleic acid extraction and the real-time PCR process. The extraction process can be performed by manual or automated systems. Then, the pure nucleic acid will be undergone the RT-PCR steps. The amplification of a targeted genome can be monitoring during the PCR as the real-time process, not at its end, as in conventional PCR.[4, 7] Thus, the RT-PCR primer of SARS-CoV-2 genomes will be matching the targeted genomes in the patients' respiratory specimens and amplified in each cycle of RT-PCR. The production of SARS-CoV2 genomes will be detected by fluorescence detection at the receptors as a real-time point. It was reported as a semiquantitative product called cycle threshold (Ct) value. The low number of Ct value represented high amount or high concentration of the viral load in the specimens. The RT-PCR assays were usually detected 2 - 3 specific genes in SARS-CoV2 genome along with the internal control gene. The medical technologists need to check the Ct standard curve for each RT-PCR process (Figure 2) along with the interpretation table (Table 1) before announced the final report.[4, 7-9] Although the RT-PCR has the highest sensitivity for SARS-CoV-2 detection and lowest limit of detection (usually less than 100 of RNA copies). The RT-PCR process required a high level of laboratory safety standard and well-trained laboratory staff. The laboratory must be certified for biosafety level (BSL) 3 or BSL 2 plus facilities. For all aerosolized procedures such as specimen preparations need to perform in the biosafety cabinet. The overall process of RT-PCR for SARS-CoV-2 takes around 4 - 6 hours. However, the laboratory TAT may be longer and depend on the process of specimen collection and laboratory workflow. The specimen collection also required trained-personal with full PPE used.[7-9] Currently, URT specimens especially the NP swabs (NP swabs only or NP swabs plus oropharyngeal-OP swabs) give the highest sensitivity on SARS-CoV-2 detection by RT-PCR compared to other URT samples.[7-9] In some patients with clinical of COVID-19 pneumonia, LRT specimens such as bronchoalveolar lavage (BAL) fluid and sputum maybe required for diagnosis.[4, 7-9] From 3 cohort studies, LRT specimens have slightly higher sensitivity 0.89 (95%CI: 0.84 to 0.94) compared to URT specimens with the sensitivity of 0.76 (95% CI: 0.51 to 1.00[59, 66, 67] and the same specificity of 1.00 (95%CI: 0.99 to 1.00).[38-40] The IDSA panel suggested performing a single viral RNA test in symptomatic individuals with a low clinical suspicion of COVID-19 (single test sensitivity of 0.71 (95%CI: 0.65 to 0.77) vs repeat test the sensitivity of 0.88 (95%CI: 0.80 to 0.96).[9, 41, 42]

Other specimens for RT-PCR

Saliva, OP swabs and nasal swabs were evaluated for alternative URT specimens for RT-PCR of SARS-CoV-2 because of the easier specimen collection process compared to NP swabs. The patients may perform self-specimen collection or under-supervision by the health care providers. However, the results showed lower sensitivity of RT-PCR for SARS-CoV2 detection when using other URT specimens compared to NP swabs including; saliva (0.90, 95%CI: 0.85 to 0.93), OP (0.76, 95%CI: 0.58 to 0.88), and NP (0.89, 95%CI: 0.83 to 0.94) and high specificity (0.98, 95%CI: 0.93 to 1.00).[26-37] In addition, pooled RT-PCR of NP swabs or saliva specimens (the combination of 4 URT specimens for single RT-PCR assay) may be applied for cost saving. If the RT-PCR assay detected SARS-CoV2 genome, the individual RT-PCR assay for each specimen will be performed. The indication for the pooled RT-PCR of SARS-CoV-2 is usually for active case finding in the outbreak evaluation. Nevertheless, the laboratory process is more

Table 1 Example of RT-PCR for SARS-Co-V-2 interpretation

ORF1ab gene (FAM)	N gene (ROX)	Internal control (HEX)	Negative control	Positive control	Interpretation
+	+	+/-	-	+	SARS-CoV-2 Positive
-	-	+	-	+	SARS-CoV-2
+	-	+/-	-	+	SARS-CoV-2
-	+	+/-	-	+	SARS-CoV-2
+	+	+	+	+	Experiment fail
-	-	-	-	-	Experiment fail

\+ : Amplification curve
\- : No amplification curve
* The single-gene amplification or even random positive results is suggestive of a) low amount of RNA template of the sample near or below the detection limit of the reactions b) mutation in the nucleic acid sequences targeted c) slightly different amplification yield of the targets regions, or d) other factors.
** If N target only is positive, the interpretation should be SARS-CoV-2 Presumptive Positive and additional confirmatory testing should be conducted by the reference laboratory if clinically indicated, to differentiate between SARS-CoV-2 and other animal coronaviruses currently unknown to infect humans.

concerned for possible of viral RNA contamination in other specimens.[43-48]

Other types of NAATs for SARS-CoV-2 detection

Rapid RT-PCR: In order to decrease complexity of traditional RT-PCR for SARS-CoV2 procedure and TAT, rapid RT-PCR were developed as commercial assays using automated systems for point of care (POC) testing. Currently, the rapid RT-PCR have been improved for the better performance and detected at least 2 genes of SARS-CoV-2. The previous studies have been reported the comparable of sensitivity of rapid RT-PCR and standard laboratory-based NAATs with the sensitivity of 0.98 (95%CI: 0.95 to 1.00) and the specificity of 0.97 (95%CI: 0.89 to 0.99).[14-25] The advantage of rapid RT-PCR is likely for emergency cases which required immediate results. However, the lower limit of detection of rapid RT-PCR varies by the assays (from 100 - 1000 copies/test) and required process of laboratory validation.

Multiplex PCR: In addition to the rapid RT-PCR for SARS-CoV-2, the commercial assays were developed and evaluated for other respiratory pathogens detection. The SARS-CoV-2 detection was add-on in the multiplex PCR assays which usually included common respiratory viruses such as respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, humanmetapneumo virus, rhino/enterovirus, and atypical bacteria (*Bordetella* spp., *Legionella* spp., and *Mycoplasma* spp.). In some assays may add-on common bacterial pathogens detection (*Staphylococcus aureus* and *Klebsiella pneumoniae*) and common nosocomial pathogens (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*). The advantage of multiplex PCR was able to detect co-infection with rapid TAT.

Some considerations of multiplex PCR are higher cost and lower sensitivity compared to the traditional RT-PCR.[17, 20, 22, 23] There were limited data on co-infection of other respiratory pathogens in COVID-19 patients.[49, 50] Lansbury et al.[49] performed meta-analysis of other respiratory infection in COVID-19 patients (30 studies, 3834 cases). RSV was the most prevalent respiratory viral co-infection (16.9%) followed by influenza A virus (15.5%). Bacterial co-infection was found 7% in hospitalized patients and 14% in ICU patients. Recently, fungal infections (*Aspergillus* spp., *Candida* spp., and Mucormycosis) in COVID-19 patients have been frequently reported especially in severe cases with poorly controlled diabetes and high-dose steroid treatment.[49-57]

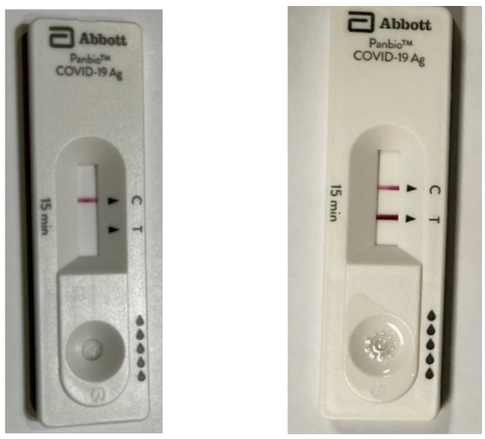
Furthermore, other types of NAATs for SARS-CoV-2 detection such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), CRISPR (clustered regularly interspaced short palindromic repeats), and molecular microarray assays are under development and validation of clinical performance.[58-60]

RT-PCR and duration of culturable SARS-CoV-2

Currently, RT-PCR for SARS-Co-V-2 is not recommended to repeat during hospitalized patients with COVID-19. The result of not detected PCR is not an indication for off isolation or discharge patients.[7-9, 61] Previous studies have been described the correlation between semiquantitative detection of the SARS-CoV-2 viral loads (Ct values) and duration of culturable SARS-CoV2 which was referred to the infectious of the virus. The reduction of infectiousness of the virus was correlated to 1) increased number of days that have elapsed since symptom onset and resolution, 2) decrease in viral load in respiratory secretions an increase in neutralizing antibodies.[32, 61-64] Singanayagam et al, reported a retrospective study of the infectiousness duration and correlation with RT-PCR viral load (Ct vales) in COVID-19 patients (324 samples) in United Kingdom between January and May 2020.[63] The viral load in the URT specimens peak around symptom onset and infectious virus persists for 10 days in mild-to-moderate coronavirus disease. The Ct values correlate strongly with cultivable virus. Probability of culturing virus declines to 8% in samples with Ct > 35 and to 6% 10 days after symptoms onset. Kim et al, also reported a prospective study of the infectiousness duration and correlation with Ct values in COVID-19 patients (21 patients, 165 samples) in South Korea, between February and June 2020.[64] Viral loads were determined with the Ct values for the N gene. Viral culture was positive only in samples with a Ct values ≤ 28.4 and during 12 days after symptom onset. In Thailand, MOPH has been announced the recommendation for COVID-19 patients to be hospitalized for 10 - 14 days without repeated NP swabs.[65] At Thammasat University Hospital, the preliminary data (35 patients) also showed the same results of prolonged presence of viral RNA (ranged 10 - 43 days, means 20 days). The Ct values and presence of viral RNA does not corelate with severity of disease.[66] Recently, Rhee et al, reviewed evidence-to-date on the duration of infectivity of SARS-CoV-2 and public health recommendations on discontinue isolation precautions.[61] Many COVID-19 patients have persistently positive RT-PCR for weeks to months following clinical recovery. SARS-CoV-2 appears to be most contagious around the time of symptom onset and infectivity rapidly decreases thereafter to near-zero after about 10 days in mild-moderately ill patients and 15 days in severely-critically ill and immunocompromised patients. The longest interval associated with replication competent virus thus far is 20 days from symptom onset. Currently, a positive NAAT diagnostic test should not be repeated within 90 days, since people may continue to have detectable viral RNA after risk of transmission has passed.[7-9, 61, 63, 64]

2. Antigen testing

Rapid antigen test for COVID-19 used the less complex diagnostic method called lateral flow immunoassays (LFI) in order to detect the SARS-CoV-2 viral protein (antigens) in the respiratory specimens (NP swabs) (Figure 3). LFI have been used for other rapid tests and POC tests such as pregnancy test and streptococcal antigen test. Although the patients are still required the NP swabs. The TAT of the rapid antigen test was only 30-60 mins and cheaper costs compared to NAATs.[7-9, 67, 68] However, rapid antigen test has less sensitivity compared to NAATs due to no amplification process. In addition, the false-positive results may occur in patients with other respiratory viral infections. Currently, data on rapid antigen test clinical performance is still limited.[67-72] Chimayo et al, reported clinical performance of rapid SARS-CoV-2 antigen test (Standard Q®) in 454 patients with early case exposure with clinical of pneumonia and pre-operative screening at the tertiary care hospital in Thailand.[67] Fifty-nine patients with positive antigen test had symptoms of pneumonia and Ct value of RT-PCR were less than 26 with the sensitivity 0.98 (95%CI: 0.91 to 0.99) and the specificity 0.99 (95%CI: 0.97 to 0.99). Thus, WHO, CDC and IDSA have recommended rapid antigen test for screening in high risk and early contact cases at the time of peak viral loads. In the patients with positive for rapid antigen test was required RT-PCR for SARS-CoV-2 confirmation. The advantage of rapid antigen tests included short TAT and available as POC testing. The rapid identification of infected people by using rapid antigen test would help us to prevent further viral transmission in the community.[7-9, 69]



Negative **Positive**

Figure 3 Example of rapid antigen tests for SARS-CoV-2.

3. Serology (antibody testing)

There were several serological tests for COVID-19 which detect antibodies produced by the patients with SARS-CoV-2 infections. For example, 1) rapid antibody test (non-quantitative assays) 2) (semi)quantitative or quantitative assays such as enzyme-linked such as immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA) 3) viral neutralizing antibody have indications for testing and limitations. Implementing serological assays in the clinical laboratory is required validation process. Most of these studies show no advantage of IgM over IgG, as IgM does not appear much earlier than IgG.[7, 73] The additional role of IgA testing in routine diagnostics has not been established.[74] For confirmation of a recent infection, acute and convalescent sera (14 days after initial sera collection) must be tested using a validated (semi) quantitative or quantitative assay and neutralizing antibody. Maximum antibody levels are expected to occur in the third/fourth week after symptom onset (Figure 4).[7, 73, 74] Previous studies have been reported the seroconversion (positive antibody based on viral neutralizing antibody or quantitative titer) at 2-3 weeks after infection. Serology should not be used as a standalone diagnostic test for clinical diagnosis or contact tracing purposes in the patients with early exposure. Antibody interpretations should be reviewed by an expert and are dependent on several factors including the timing of the disease, the epidemiology and prevalence of disease.[7, 72-75] Thus, serological tests for COVID-19 was recommended only for some indications; 1) symptomatic patients with negative results from NAATs after 2 weeks of clinical disease, 2) epidemiological study or seroprevalence testing and 3) patients who suspected Multisystem Inflammatory Syndrome in Children (MIS-C).[73]

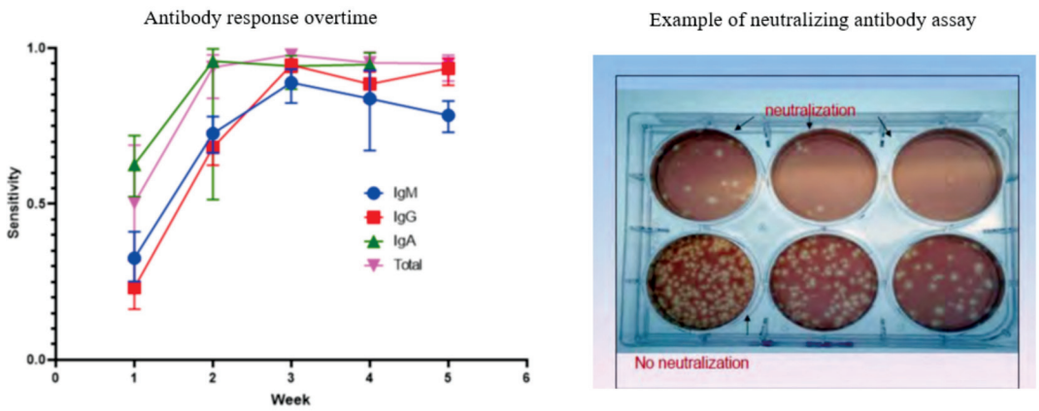


Figure 4 Antibody response for SARS-CoV-2 infections overtime and example of neutralizing antibody assay.

Rapid antibody tests: LFI assays for SARS-CoV-2 antibody detection is usually used as POC tests which provided only positive or negative results without antibody titers.[74] The advantage of rapid antibody tests are rapid TAT, less complexity of testing procedure and cheaper than NAATs and rapid antigen tests. However, the rapid antibody tests have high false-negative result especially in early contact cases. In addition, the interpretation of the test results need to be perform by experts or laboratory technicians and require laboratory validation process due to the varies or different results based on the difference commercial assays (Figure 5). The preliminary data of rapid antibody test validation in clinical patients setting at Thammasat University Hospital during March to May 2020 also showed varies in time for antibody detection in range of 9 - 25 days after onset of symptoms or clinical exposure (n = 47).[66]

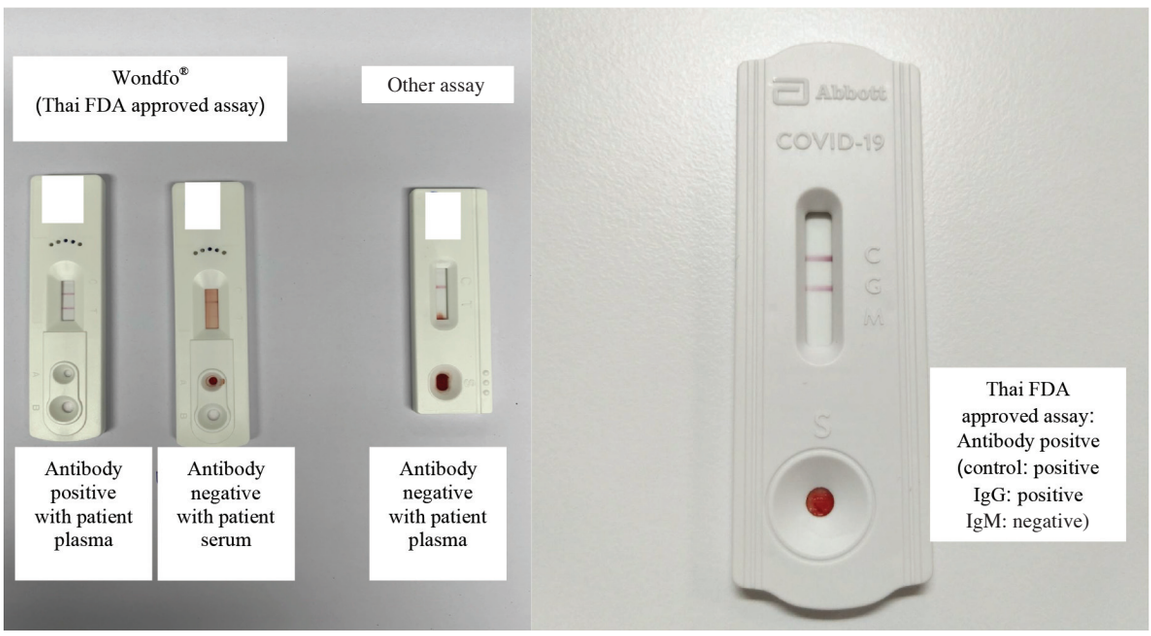


Figure 5 Example of Rapid Antibody Test for SARS-CoV-2 (varies test results by different assays).

Quantitative tests: Commercial and non-commercial tests measuring binding antibodies (total immunoglobulins (Ig), IgG, IgM, and/or IgA) used various detection techniques including LFI, ELISA, and CLIA have become available and validated.[7, 73, 75-80] The performance of serological assays varies in different clinical settings based on severity of disease, age group, timing of testing and the target viral protein. Antibody detection tests for coronavirus may also cross-react (false positive test) with other pathogens such as other human coronaviruses and patients with pregnancy and autoimmune diseases.[81-84]

Virus neutralization assays: It is considered to be the gold standard test for detecting the presence of functional antibodies (Figure 4). Neutralization of SARS-CoV-2 has been reported for antibodies that bind to Spike, Membrane and Envelope proteins and remained as neutralizing antibody targets. Rapid development of neutralizing antibody response to Spike correlates with viral immunity or seroconversion. However, the viral neutralization assays or surrogate viral neutralization tests require highly skilled staff and BSL-3 laboratory facilities for viral culture.[7, 73]

In current recommendation, a reliable diagnosis of COVID-19 infection based on patients' antibody response will often only be possible in the recovery phase. Therefore, serology test is not a suitable replacement for virological assays to inform contact tracing. Furthermore, the presence of antibodies that bind to SARS-CoV-2 does not guarantee that they are neutralizing antibodies or protective immunity.[7, 8, 73, 77]

4. Viral isolation

Virus isolation is a traditional diagnostic test to confirm the novel viral pathogens. However, it is not recommended as a routine diagnostic procedure due to the complexity of the procedures and laboratory safety concern. All procedures involving viral isolation in cell culture require well-trained staff and BSL-3 laboratory facilities.[7, 8, 83]

5. Genomic sequencing

Genomic sequencing for SARS-CoV-2 have been used to investigate the dynamics of COVID-19 epidemiology especially on outbreak investigation including changes in the size of an epidemic outbreak, disease spreading and routes of transmission. Moreover, genomic sequences help epidemiologists for further understand about genetic linage, genetic mutation and variant of the virus which impact on diagnostic tests, treatments, and vaccinations.[7-9, 84] The established nomenclature systems for naming and tracking SARS-CoV-2 genetic lineages by GISAID, Nextstrain and Pango are currently use by scientists and in scientific research. At the present time, this expert group convened by WHO has recommended using labeled using letters of the Greek Alphabet for SARS-CoV-2 Variants of Interest (VOI) and Variants of Concern (VOC) (Table 2).[85]

The process of genomic sequencing for SARS-CoV-2 was required reference laboratories and expertise to perform. Nowadays, the technology of genomic sequencing has been advanced and varies based on run-time, costs, complexity of procedure, data processing, rate of data production, and sequencing accuracy. Conventional sequencing (Sanger sequencing) can be used to sequence individual fragments (such as mutation confirmation up to 1000 bp) in separate reactions. In contrast, the next-generation sequencing platforms (Illumina, IonTorrent, and Oxford Nanopore Technologies) are more appropriate for whole genome sequencing with multiple samples sequenced together in a single run. However, the high cost and volume of the work required for genomic sequencing has limit the genomic sequencing for SARS-CoV-2 for the reference laboratories.[1, 7, 84, 86]

Table 2 SARS-Co-V-2 Variants of Concern (update 1 July 2021)

WHO label	Pango lineage	GISAID clade/lineage	Nextstrain clade	Additional amino acid changes monitored*	Earliest documented samples	Date of designation
Alpha	B.1.1.7	GRY (formerly GR/501Y.V1)	20I (V1)	+S:484K +S:452R	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351 B.1.351.2 B.1.351.3	GH/501Y.V2	20H (V2)	-	South Africa, May-2020	18-Dec-2020
Gamma	P.1 P.1.1 P.1.2	GR/501Y.V3	20J (V3)	+S:681H	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2 AY.1 AY.2	G/478K.V1	21A	-	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021

Discussion

Laboratory tests play an important role for COVID-19 diagnosis. The diagnostic key point is not only based on the type of laboratory test, but also the appropriate testing during the course of disease. In the early course of COVID-19 infection, RT-PCR of the NP swab is the gold standard test for SARS-CoV-2 detection due to the highest sensitivity and specificity of the tests during the first 2 weeks after the exposure. There are several types of NAATs such as traditional RT-PCR, rapid PCR, and multiplex PCR which may have difference TAT, diagnostic performance, and limitations (Table 3). Rapid antigen test has less complexity of the testing procedure with faster TAT compared to NAATs. Serology tests for COVID-19 are recommended only for some indications. Viral isolation and genomic sequencing testing are not routinely used for COVID-19 diagnoses, but they are important for the study of viral mutation and outbreak investigation.

Table 3 Summary of laboratory diagnostic testing for COVID-19

	NAATS (RT-PCR)	NAATS (Rapid PCR/ Multiplex PCR)	Antigen test	Serology (Antibody test)
Testing indication(s)	Early / current infection	Early / current infection	Current infection (high prevalence/ symptomatic patients)	1) Symptomatic patients with negative NAATs after 2 weeks of infection 2) Epidemiological study 3) Multisystem Inflammatory Syndrome in Children (MIS-C).
Target	Viral RNA	Viral RNA	Viral antigen	Antibody
Specimen(s)	Nasopharyngeal, oropharyngeal, sputum, saliva	Nasopharyngeal, oropharyngeal, sputum, saliva	Nasopharyngeal	Blood, serum
Sensitivity	High	Varies by tests (moderate-high for point of care tests)	Varies (moderate-to-high at times of peak viral load)	High (after 2 weeks of infection)
Specificity	High	High	Varies (high at times of peak viral load)	High (after 2 weeks of infection)
Limit of detection	< 100 RNA copies	Varies by tests (< 100 - 1000 RNA copies)	Varies	N/A
Test complexity	Varies by tests (generally complex)	Relatively easy to use	Relatively easy to use	Varies by tests (Neutralizing antibody-very complex, ELISA – moderate complexity, rapid antibody/ lateral flow assay – low complexity)
Point of care test (POC test)	No	Yes	Yes	Varies by test
Turn-around time (TAT)	Procedure time: 4 - 6 hours (TAT included specimen collections and laboratory work flow maybe 1 - 3 days)	Procedure time: 2-6 hours	Procedure duration: 15 minutes to 30 minutes	Ranges from 15 minutes to 30 minutes for rapid antibody, ELISA – 1 - 2 hours, neutralizing antibody for weeks

Table 3 Summary of laboratory diagnostic testing for COVID-19 (Cont.)

	NAATS (RT-PCR)	NAATS (Rapid PCR/ Multiplex PCR)	Antigen test	Serology (Antibody test)
Cost	Moderate (~$50 - $100/test)	Moderate (~$75 - $250/test)	Low (~$5 - $50/test)	Varies by test
Advantages	- Most sensitive - Does not need to be repeated to confirm results	- Sensitive - Short TAT - Co-infection detection (multiplex PCR)	- Short TAT - POC tests assist prevention of disease transmission prevention	Epidemiological study and as in the indications
Disadvantages	- Longer TAT - Higher cost - A positive NAAT should not be repeated within 90 days (detectable RNA after risk of transmission has passed)	- Higher cost - A positive NAAT should not be repeated within 90 days (detectable RNA after risk of transmission has passed)	- Need confirmatory testing (NAATs) - Less sensitive compared to NAATs, especially among asymptomatic people	Do not use as standalone test for early infection

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