# Diagnosis of COVID-19: the present and the future

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## Abstract

Increased requirement for diagnostic tests has posed a significant burden on the healthcare systems throughout the world as the COVID-19 pandemic evolves. The recommended test at present for a diagnosis of SARS-CoV-2 infection is the reverse transcriptase polymerase chain reaction (RT-PCR) performed on a relevant clinical sample. Rapid point of care molecular assays is available for use and these could accelerate the diagnostic process and help triage patients at emergency settings in the future.

**Key words:** SARS-CoV-2, COVID-19, Diagnosis, testing, RT-PCR

## Introduction

The COVID-19 pandemic due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has imposed a major burden on the healthcare sector in most countries including Sri Lanka. Testing strategies vary around the world. Countries such as South Korea, Taiwan and Hong Kong have adopted aggressive screening strategies for suspected SARS-CoV-2 cases while many countries have faced limitations in testing capacity[1,2]. Performing diagnostic testing for SARS-CoV-2 has become further hampered due to limitations in reagents, scarcity of nasopharyngeal swabs, reduced availability of viral transport media required for collection and transport of specimens and the lack of trained staff to perform the diagnostic tests. Rapid molecular diagnostic tests have been developed and given emergency use authorization by the U.S. Food and Drug Administration[3]. Such an expedited approval is different to the normal procedure and the performance of these tests would need better evaluation in the future. In addition, it is a significant challenge for the companies to meet the overwhelming global requirement as the need for diagnostics including supportive tests for these patients continue to increase with the surge of new cases detected.

The need for diagnostic testing has significantly increased in Sri Lanka with the change in case definition and the shift in the surveillance strategy. The number of tests performed increased from approximately 200-250 tests/day in early April to 1100-1900 tests/day by the end of April 2020 (data from Ministry of Health). The number of laboratories performing RT-PCR have increased from a handful at the beginning of February 2020 to 15 centres as at 06th May 2020 (data from Ministry of Health). This review summarises the investigations used at present for diagnosis of SARS-CoV-2 infection, their applicability and the newer tests in the pipeline.

## Diagnosis of SARS-CoV-2 infection

A rapid and an accurate diagnosis is essential to identify suspected SARS-CoV-2 infected cases early, to contain the outbreak by appropriate quarantine measures and to employ contact tracing for surveillance. The gold standard test to diagnose SARS-CoV-2 is a RT-PCR.

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### Diagnostic test: RT-PCR

SARS-CoV-2 is a RNA virus belonging to genus beta-coronavirus in the coronavirus family. Virus contains 4 structural proteins (membrane, envelope, spike and nucleocapsid) and several non-structural proteins[4]. Currently recommended RT-PCR protocols identify a minimum of 2 targets of the SARS-CoV-2 genome with one being specific for the SARS-CoV-2[5,6]. Some kits identify one target sequence specific to SARS-CoV-2 while the second target is a nucleic acid sequence of beta-coronavirus. The amplification of the nucleic acid during PCR enables identification of low viral copies in the clinical sample. Alternatively, centres for disease control also recommends doing RT-PCR to identify a target sequence of beta-coronavirus which is followed by sequencing of the viral genome to confirm the diagnosis of SARS-CoV-2.

Viral load peaks at the time of onset of symptoms in SARS-CoV-2 similar to influenza[7] in contrast to Severe acute respiratory syndrome (SARS) which peaks at approximately 10 days or Middle East Respiratory Syndrome (MERS) which peaks at 2 weeks[7]. Available data suggest that infected persons shed the virus from their respiratory tracts beginning in the late-incubation period until recovery allowing RT-PCR performed on a relevant sample to detect viral nucleic acid early in the course of the disease[8]. Viral RNA from clinical specimens can be detected for several weeks following clinical recovery[9,10]. The estimated sensitivity of the RT-PCR varies from 66% to 80%[11]. False negative results have been a concern and repeating the sample is recommended when there is a high index of clinical suspicion. In a study by Shen et al., repeating RT-PCR in a nasopharyngeal (NP) swab for a second time, increased the detection rate of SARS-CoV-2, 1.27-fold (35.5%/27.9%) and repeating the test three times increased the detection rate 1.43-fold (39.9%/27.9%) compared to performing the test once[12]. The increase in detection rate of SARS-CoV-2 was not seen in some sub groups by repeating RT-PCR for three times compared to once and the detection rate did not improve when the test was repeated beyond three times[12]. The current clinical practice guideline issued by the Ministry of Health in Sri Lanka advises to repeat the RT-PCR in 24 hours if a patient with a high index of suspicion has a negative result[13].

### A false negative RT-PCR result: What does it mean?

A false negative RT-PCR result could be due to many reasons and not necessarily related to the test procedure. Detection of viral RNA will depend on the viral load, sampling quality and obtaining the relevant sample, proper transport of the clinical specimens and RT-PCR testing process[12]. Intermittent viral shedding and low viral load can result in a negative RT-PCR result. Adhering to the proper technique of collecting a NP or an oropharyngeal (OP) swab is of paramount importance. In addition, proper transport of the specimen and maintaining a temperature of 4°C until processing is important to preserve viability of the virus. Finally, inadequate extraction of RNA, the presence of PCR inhibitors or using a PCR kit with low sensitivity could affect the performance of RT-PCR.

### Rapid tests: what the future holds?

Despite the high accuracy and reliability, the RT-PCR could take 4-6 hours for sample processing, RNA extraction and the RT-PCR itself. In addition, trained laboratory technicians and appropriate laboratory facilities are needed to perform a RT-PCR. A rapid diagnosis (within 1 hour) of SARS-CoV-2 is useful to triage patients in emergency settings and to minimise healthcare worker exposure risk. Thus, there is much enthusiasm on producing a rapid diagnostic test in this setting and several products have been developed using molecular methods or antigen detection methods.

### a) Self-enclosed rapid molecular assay systems:

ID NOW™ (Abbott), cobas® Liat® (Roche), BioFire FilmArray (bioMérieux) and GeneXpert® (Cepheid) have a self-enclosed system which has integrated the RNA extraction, amplification and detection in to a cartridge[1,9]. Simple workflow of these assays allow minimal sample handling and does not require an intensive training for the laboratory technicians. The clinical specimen in viral transport medium (VTM) is transferred into the cartridge in a class II biosafety cabinet and placed in the relevant instrument for processing. The cartridge is "sealed" and it does not require manual processing of the sample further. These cartridge-based tests are ideal for point of care testing. According to published literature, these can be used at places without a class II biosafety cabinet[9]. In such situations, the person who collects the specimen could directly transfer the specimen into detection cartridges at bedside. The collector will be equipped with appropriate protective gear and the closed cartridge will be safely placed on instrument for testing afterwards[9]. The drawback is that the relevant devices should be available to perform the test. However, GeneXpert machines are available at several

chest clinics throughout Sri Lanka, providing an ideal opportunity to use this rapid assay for SARS-CoV-2 diagnosis if the need arises. The biggest limitation in using this test is that cartridges and reagents will only be available after August 2020.

*b) Rapid antigen based assays:*

Lateral flow assays that detect viral antigens are currently under development. However, the sensitivity will be the biggest concern considering the variable viral load seen in SARS-CoV-2 infection. The experience from antigen detection tests developed for influenza suggest that sensitivity of these tests could be a major drawback[9]. The N-antigen detection rapid kit developed by Diao et al. which takes 10 minutes to perform providing a quick method of viral protein detection had a sensitivity of 68% in the tested samples when the RT-PCR of NP swab was taken as the reference standard[14].

**The use of serological assays**

We are still learning the viral dynamics including the host response to SARS-CoV-2 infection.

Studies have consistently reported that antibodies develop approximately after 10 days[7,15]. SARS-CoV-2 specific IgM and IgG antibody detection in 285 patients with COVID-19 revealed that development of IgG and IgM occurred simultaneously or IgG antibody followed by IgM[16]. Interestingly, most studies reveal that SARS-CoV-2 had an earlier IgG response than IgM when tested. This particular study revealed that all tested SARS-CoV-2 positive patients had detectable antibodies after 17-19 days of symptom onset[16]. The median seroconversion time for both IgM and IgG was 13 days and the seroconversion time for IgM in 94.1% patients reached 20-22 days[16]. As such, SARS-CoV-2 antibody detection would unlikely to be of benefit in acute case detection. However, it will be useful for identifying community prevalence, immunity assessment of healthcare workers and also in surveillance (identifying asymptomatic close contacts who would be missed by RT-PCR)[9,16]. In the study by Long et al., there were 7/164 (4.3%) close contacts of SARS-CoV-2 infected patients who were positive for antibodies at 30-days following exposure but had a negative RT-PCR during surveillance[16]. It is also important to validate these serological tests in the local setting due to possible cross-reactivity with non-SARS-CoV-2 coronaviruses.

**Clinical samples recommended for diagnostic testing**

The sample to use in an infected individual to detect SARS-CoV-2 should be guided by the clinical presentation. Collection of the appropriate sample at the correct time has a major impact on the final PCR result. Specimens used to identify SARS-CoV-2 are upper respiratory tract samples such as nasal swabs (mid-turbinate swab or anterior nasal swab), nasal washes, NP swabs and OP swabs[6]. The lower respiratory tract samples are sputum, broncho-alveolar lavage fluid and endotracheal aspirate. Saliva has also been suggested as an appropriate sample to detect SARS-CoV-2 in several studies. There has been high correlation between virus detection from NP swabs to saliva[7] and the viral load was highest in saliva during the first week of illness[7].

NP swabs and/or OP swabs are recommended in early infection[9]. The swab used for collection should be a synthetic fibre swab with a plastic or wired shaft. Cotton, calcium alginate or wood can give rise to false negative results in the RT-PCR[5,6]. OP swabs collected from the posterior pharynx can be transported similar to NP swabs and should be placed in the same vial for a higher sensitivity[5]. A retrospective analysis of data in 353 patients in Wuhan who had a NP swab and an OP swab simultaneously revealed that NP swabs had a higher positive rate than OP swabs (67/353 vs 27/353, p<0.0001)[17]. Nasal swabs also had lower sensitivity of 89% compared to NP sampling[18]. Viral loads and the rate of positivity are reported to be higher in sputum than throat swabs, NP/OP swabs[8,19]. Therefore, it is advisable to collect sputum if the patient has lower respiratory tract symptoms and producing sputum. Tracheal aspirate or broncho-alveolar lavage fluid will be an appropriate sample in intubated patients[11,13]. NP swabs are useful in asymptomatic patients to detect SARS-CoV-2.

Most case series around the world report that viraemia is not a common occurrence in this SARS-CoV-2 infection, rendering serum an inappropriate sample for virus detection[19]. Urine samples cannot be used for diagnosis of SARS-CoV-2 as viral nucleic acid was not detected in urine by RT-PCR[1,19].

**The usefulness of imaging in SARS-CoV-2 infection**

Computed tomography (CT) chest was found to be sensitive in detecting SARS-CoV-2 pneumonia in a

large study correlating RT-PCR and CT chest in Wuhan, China. Chest CT images of 308 patients with negative throat swab RT-PCR had radiological features suggestive of SARS-CoV-2 while 147 (48%) of them were classified as highly likely to be COVID-19 based on the clinical symptoms, CT features and serial radiological changes[11,20]. These data suggest the usefulness of CT Chest in diagnosing SARS-CoV-2 in RT-PCR negative cases with lung consolidation. Most commonly reported changes in chest CT of SARS-CoV-2 patients include ground glass opacities (77.8-100%) occurring in a peripheral distribution, fine reticular changes and vascular thickening[1]. These changes were commonly reported to be seen bilateral than unilateral in literature (11-100%)[1]. Conventional chest radiograph had low sensitivity of 59% in detecting SARS-CoV-2 related lung consolidation[11].

## Overcoming the challenges

Biggest challenge to effective testing worldwide has been lack of reagents necessary for RT-PCR and the shortage in swabs and viral transport media (VTM) for sample collection. Sri Lanka has faced the same problems due to interruption to supply chain. It is important to have a regulatory mechanism in place to ensure an uninterrupted supply. Using a self-collected posterior pharyngeal saliva sample, the use of self-collected sputum or use of nasal washes would overcome the issue of lack of NP swabs. However, it is important to give proper instructions to the patient and also validate the rate of positivity of samples such as saliva prior to use. Samples can be transported in normal saline, phosphate-buffered saline or Amies transport medium in the event of lack of VTM. The Centers for Disease Control and Prevention, USA has provided the standard operating procedure for in-house preparation of VTM which provides a useful guide for local preparation of solution.

## Conclusion

The performance and accuracy of the RT-PCR depends on the viral load, collection of an appropriate clinical sample with proper transport and appropriate processing of the specimen during the PCR. Serological assays would unlikely to be beneficial in diagnosing SARS-CoV-2 in acute infection but these tests might provide useful information on community prevalence and help assess the true extent of the outbreak. Rapid molecular assays such as GeneXpert and other point of care tests could be useful to provide a rapid diagnosis with minimum use of skilled laboratory expertise in the future.

## References

1. Cheng MP, Papenburg J, Desjardins M et al. Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus-2: A Narrative Review. *Annals of Internal Medicine* 2020. https://doi.org/10.7326/M20-1301

2. Korean Society of Infectious Diseases, Korean Society of Pediatric Infectious Diseases, Korean Society of Epidemiology, Korean Society for Antimicrobial Therapy, Korean Society for Healthcare-associated Infection Control and Prevention, and Korea Centers for Disease Control and Prevention. Report on the Epidemiological Features of Coronavirus Disease 2019 (COVID-19) Outbreak in the Republic of Korea from January 19 to March 2, 2020. *Journal of Korean Medical Sciences* 2020 Mar 16; **35**(10): e112

3. U.S. Food and Drug Administration. Emergency Use Authorization. 2020 [cited 2020 04.5.2020]; Available from: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization.

4. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes and Metabolic Syndrome* 2020; **14**(4): 407-12.

5. Iyer M, Jayaramayya K. Subramaniam MD, et al. COVID-19: an update on diagnostic and therapeutic approaches. *BMB Reports* 2020; **53**(4): 191-205.

6. Centers for Disease Control and Prevention. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). 2020 [cited 2020 7.5.2020]; Available from: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

7. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infectious Diseases* 2020; March 23. https://doi.org/10.1016/ S1473-3099(20)30196-1

8. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infectious Diseases* 2020; Feb 24. DOI:10.1016/S1473-3099(20)30113-4

9. Tang Y, Schmitz J, Persing DH, Stratton CW. The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. *Journal of Clinical Microbiology* 2020; April 3. doi: 10.1128/JCM.00512-20. [Epub ahead of print]

10. He Xi, Lau EHY, Peng W, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine* 2020; **26**(5): 672-5.

11. Pascarella G, Strumia A, Piliego C, et al. COVID-19 diagnosis and management: a comprehensive review. *Journal of Internal Medicine* 2020; April 29. doi.org/10.1111/joim.13091

12. Shen N, Zhu Y, Wang X, et al. Characteristics and diagnosis rate of 5,630 subjects receiving SARS-CoV-2 nucleic acid tests from Wuhan, China. *JCI Insight*. 2020. https://insight.jci.org/articles/view/137662

13. Ministry of Health Sri Lanka. Provisional Clinical Practice Guidelines on COVID-19 suspected and confirmed patients. Version 5. 30th April 2020. Available from www.epid.gov.lk. Accessed on 04.05.2020.

14. Diao B, Wen K, Chen J, et al. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. MedRxiv, 2020: p.2020.03.07. 20032524. https://doi.org/10.1101/2020.03.07.20032524

15. Zhao J, Yuan Q, Wang H et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clinical Infectious Diseases* 2020; March 3. DOI: 10.1093/cid/ciaa344

16. Long Q, Liu B, Deng H et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine* 2020; April 29. doi.org/10.1038/s41591-020-0897-1

17. Wang X, Tan L, Wang X, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *International Journal of Infectious Diseases* 2020; **94**: 107-9.

18. Péré H, Podglajen I, Wack M, et al. Nasal swab sampling for SARS-CoV-2: A convenient alternative in time of nasopharyngeal swab shortage. *Journal of Clinical Microbiology,* 2020: p. JCM.00721-20.

19. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *Journal of the American Medical Association* 2020; March 11: E1-2. DOI: 10.1001/jama.2020.3786

20. Ai T, Yang Z, Hou H, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology* 2020; Feb **26**: 200642. DOI: 10.1148/radiol.2020200642