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Mini-Review

Updates on COVID-19 Diagnostic Testing

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ABSTRACT

Despite the significant containment efforts, the coronavirus disease 2019 (COVID-19) pandemic continues to spread in many countries with varying degrees of clinical symptoms. Thus, there is an urgent need for a collaborative approach, including accurate diagnosis, epidemiology, surveillance and prevention by using high-speed technology. The RT-qPCR (the Reverse Transcription-quantitative Polymerase Chain Reaction) assay is considered to be the gold standard for the early detection of the virus although this technique has limited application to use as a bedside test because of the technical complexity. As a result, using qRT-PCR in SARS-CoV-2 (Severe Acute Respiratory Syndrome) detection remains the most treasured because of its specificity, rapid turn-around, and reliability. Detection of IgG and IgM antibodies by various assays is also available. Moreover, these assays can show the current or past infection, vaccination responses, and identification of neutralizing antibodies titers in recovered individuals. However, the less valuable used technique in tracing viral infection, monitoring mutation and subtype classification is the viral genome sequencing. In addition, an emerging CRISPR-Cas-based assay, such as Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK), might also additionally provide an alternative for rapid and point-of-care detection. In conclusion, varied methods are available for viral genome and protein detection and the selection of specific method relies on the purposes of diagnosis and prevention or monitoring of vaccination efficacy.

Keywords: COVID-19; SARS-CoV-2; CRISPR-Cas-based assay; RT-PCR; Coronavirus disease 19

INTRODUCTION

COVID-19 is a disease caused by SARS-CoV-2 and can lead to mild and severe infections in humans. Since its first appearance in China in December 2019, pandemics are spreading rapidly around the world. Despite the efforts with the disease, the virus continues to spread in many countries with varying degrees of clinical symptoms. SARS-CoV-2 infection has an extensive range of clinical manifestations varying from asymptomatic to symptomatic consisting of respiratory symptoms, fever, shortness of breath, cough, dyspnea and viral pneumonia and in severe cases, severe acute respiratory syndrome, heart failure and renal failure [1]. Some of these symptoms are the predominant cause of COVID-19 associated death [2]. The major complications of COVID-19 are the pneumonia and acute respiratory distress which will elicit the immune responses ending up in massive uncontrolled inflammation and tissue damage [2]. Furthermore, Multisystem Inflammatory Syndrome in Children (MIS-C) was confirmed to be associated with the SARS-CoV-2 infection [3]. Thus, COVID-19 is responsible for the significant morbidity and mortality during the coronavirus pandemic and the need for the reliable rapid diagnostic method is very crucial.

Coronaviruses are enveloped, positive single-stranded RNA genome and the Coronavirinae family consists of four genera: Alpha, Beta, Gamma and Delta coronavirus [4]. Specifically, the SARS-COV-2 strain is classified into the genera of β-coronavirus with an RNA genome size of 29.9 kb [2,5,6].

There are a number of published studies that point out that the genome of SARS-CoV2 has gone through evolutionary modification and transformation at some stage of its pandemic spreading resulting in increased genetic variation and various

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mutations. It was found that, according to recent published data, the SARS-CoV-2 virus is primarily transmitted between people through inhalation or contact with contaminated droplets with an incubation period lasting for about 14 days [7-9].

This review presents an up to date and comprehensive overview on the performance of the distinct nucleic acid based and serological methods currently available for the reliable diagnosis of COVID-19. The data mentioned in this review is hoped to clinicians and clinical microbiologists to choose a suitable method for SARS-CoV-2 detection and thereby clinical management.

LITERATURE REVIEW

Diagnosis of COVID-19

Molecular testing of SARS-CoV-2: The reliable diagnostic technologies had right away evolved and advertised to assist early prognosis of COVID-19. Thus far, the most common diagnostic methods that have been commercialized are the molecular diagnostics that detect part of the viral RNA genome in the respiratory tract specimens while serological or antibody assessments detect SARS-CoV-2 particular antibodies in serum samples. The qRT-PCR starts with reverse transcription of the RNA to cDNA for PCR amplification. Next, the targeted viral gene will be extracted to undergo the PCR process. There are three highly conserved regions that have been found in the SARS-CoV-2 genome; *RdRp* (RNA-dependant RNA polymerase), *E* (Envelope) and *N* (Nucleocapsid) genes as shown in Figure 1. Generally, the molecular assays are a two-target system with one primer that universally detects variable virus including SARS-CoV-2 and the second primer exclusively detects SARS-CoV-2. The RT-PCR standard process includes three main steps: collection of the sample, lysis and amplification. The process takes about 3 hr to complete and show results [10].

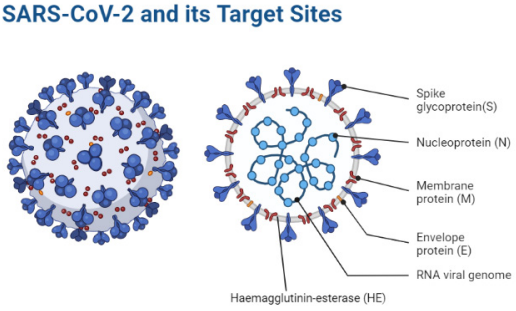

Figure 1: Diagram of coronavirus structure and its target. The virus consists of a single strand RNA that encodes all viral genes and structural proteins including Spikes glycoprotein (S), Nucleoprotein (N), Membrane protein (M) and Envelope protein (E). Some of subtypes also have Haemagglutinin-Esterase (HE) on their membrane.

For a routine workup, it's far endorsed to use a first line screening gene which is *E* gene, followed by a confirmatory *RdRp* gene [11]. A wide variety of RT-PCR primer and probe sets were authorized for SARS-CoV-2 detection through the Center of Disease Control (CDC) [10].

The whole genome structure of SARS-CoV-2 has helped the layout of specific primers and has introduced the best diagnostic protocols [2,12,13]. The high specificity and limited sensitivity of the spike region of SARS-CoV-2 using RT-PCR was first confirmed by many published reports [14]. Later, the sensitivity of this method became substantially stepped forward with the aid of using particular probes for the other viral-specific genes, such as *RdRp* in the *ORF1ab* region, *N* and *E* genes. To avoid contamination with other human RNA and to prevent the potential genetic shift of SARS-CoV-2, two genetic targets should be involved in this assay: One nonspecific target to detect other coronaviruses, and one specific target for SARS-CoV-2. The most highly sensitive target is the *RdRp* gene according to the validation study carried out in about 30 European laboratories using synthetic nucleic acid technology [15].

A novel RT-PCR assay has been proposed by Chen et al. in which a sequence of the *RdRp/Hel* could detect low viral COVID-19 load in the upper respiratory tract, plasma and saliva samples without any cross-reactivity with other common respiratory viruses [11,16]. Moreover, Peñarrubia et al. carried-out evaluation study to assess the effect of sensitivity on five different PCR based assays against the accumulated genetic variability of the SARS-CoV-2 virus. It was found that targeting more than one viral gene is very essential to avoid false negative reports due to unknown mutation especially in the COVID-19 outbreak [17].

In addition, one of the state-of-the-art biosensing systems used in COVID diagnosis is an isothermal amplification-based method such as the Loop-Mediated Isothermal Amplification (LAMP) [18]. In this method, there is a production of many viral gene copies from a single fragment at a constant temperature compared to RT-PCR that use thermal cycler coupled with fluorimetry. LAMP can limit the false negative detection of unknown variants through the use of multiple primers. It is more specific and sensitive than RT-PCR although the LAMP method requires optimization of the reaction conditions that presents a major challenge [19].

Other nucleic acid-based assays (NGS, CRISPR, SHERLOCK): Since the coronavirus is RNA virus which makes up the most variation and is the principal cause of the most infectious disease including COVID-19, the novel RNA based technologies play an important role in tracing the infections [20,21]. Thus, there is an urgent need for such technologies like Next generation Gene Sequencing (NGS) to be applied in the research and the diagnostic field. Although this approach is less competent due to its high cost, it is crucial in finding the origin and the intermediate host of SARS-CoV-2, monitoring mutation and in subtype classification of emerging variants [22,23].

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is one of the most revolutionary and genetic modification technique breakthroughs in science in which programmable nucleases enzyme (Cas9 protein) is used to predict off-target sites and to modify several genomic sites simultaneously (multiplexing) [24,25]. The RNA from Cas9 endonuclease and from the CRESPR which carries the complementary sequence will be recognized by the RNA transcripts containing the target sequence as shown in Figure 2. The formation of the later complex

switches on the cleavage activity of the enzyme and detects the target RNA through emitting fluorescing signals [26,27].

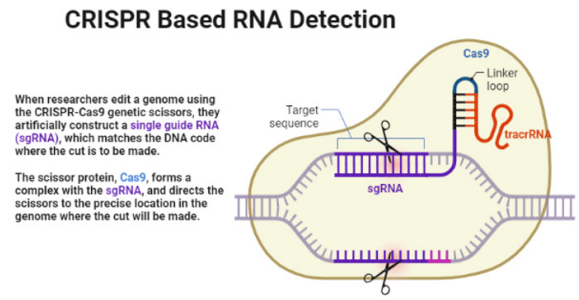

Figure 2: CRISPR based RNA detection.

A CRISPR-based diagnostic platform has been established recently to combine preamplified viral nucleic acid with CRISPR-Cas9 enzyme for specific detection of desired DNA or RNA sequences. This platform is known as Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) [28].

The SHERLOCK procedure consists of two main steps: isothermal target nucleic acid amplification and CRISPR-Cas13 nucleic acid detection. Cas13 will act as an enzyme that will carries multiple nonspecific cleavage sites after binding to a specific target. SHERLOCK will help in detecting genetic signatures of viruses without the need for complicated lab procedures. Another platform is SHERLOCK Testing in One Pot (STOP) which has been developed to combine extraction of viral RNA with isothermal amplification [29].

Serological testing for SARS-COV-2 detection

Although the molecular testing for COVID is considered as the gold standard, the diagnostic methods underlying the development of serological and immunological techniques is of a pressing need as an alternative. The production of antibodies against the SARS-CoV-2 serves as an immunity passport; in some countries; in which the individuals are assumed to be protected against reinfection. However, this plan may increase the risk of the disease transmission since till date no clear evidence supported the immunity protection from the previous infection.

A validation study carried out by Akyala et al. evaluated the diagnostic importance of different novel antibody based diagnostic tests (combined IgG and IgM test kits) from 134 confirmed COVID health care workers by RT-PCR. Compared to RT-PCR., they found that, two types of the COVID-19, IgM-IgG combined antibody kits had low sensitivity, optimal sensitivity and above average accuracy. The study concluded that, the use of rapid diagnostic kits with remarkable sensitivity, specificity and accuracy is complementary to detecting and combating SARS-CoV-2 infections [30].

A similar result of ideal analytical sensitivity and specificity was obtained by Fang and Meng in Sweden which is the minimum rate required to achieve a 90% or higher true positive rate [31]. In addition, the serology detection-based method reflects the severity of the patient's symptoms unlike the results obtained from RT-PCR results. Yu et al. evaluated the level of IgM and IgG antibodies and they found that these levels were significantly higher in severe infected patients than in mild to moderate COVID cases [32].

Different serological based tests are commercially available for COVID-19 detection such as flow immunoassay LFIA, Enzyme-Linked Immunoassay (ELISA) and Chemiluminescent Immunoassay (CLI) [33-35]. The host humoral response was triggered upon the Infection of SARS-CoV-2 ending in continuous production of a large number of specific antibodies; IgA, IgM, and IgG antibodies against SARS-CoV-2 [36,37]. These specific antibodies are more concentrated and last for a longer time than the viral antigen particles [32]. The LFIA principle based on the detection of the viral antigen once the antibody presents in the patient sample conjugated with the antigen in the manufactured kit and the results will be displayed within 5 to 15 minutes [38]. Although there are a variety of manufactured LFIA assays with varying sensitivity, the only available assay that target the highly conserved nuclear region of SARS-CoV-2 and able to detect the antigens within 15 min with specificity of 99.5% is COVID-19 Ag Respi-Strip developed by Mertens and colleagues [39,40]. Furthermore, Stieber and his colleagues reported an LFIA based analyzer using fluorescent nanoparticles as a signal to detect COVID antigens with 100% specificity and sensitivity [41].

Another option for easy and less expensive antibody test is the ELISA. The higher reproductivity and optimal sensitivity of ELISA based methods is an excellent method to detect various infections including SARS-CoV-2 [35]. Due to the enzymatic reaction, ELISA enables the quantitative analysis of antigen antibody reaction [42]. Both ELISA and CLIA are carried out in microplate well. In ELISA the results are detected by change in a color while in CLIA the results are verified by production of light in chemiluminescence analyzer [33,34].

Table 1 elucidates the practical principles of the three most widely used serological assays: The Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescence Immunoassay (CLIA) and Lateral Flow Immunoassay (LFIA).

Table 1: The practical principal differences between ELISA, CLIA and LFIA.

	ELISA	CLIA	LFIA
Sample	Blood	Blood	Blood
Analysis platform	Microplate	Microplate	Paper based
Detection time	2-8 hr	0.5-2 hr	5-15 min
Operation process	Complicated	Relatively simple	Simple
Reaction product	Chromogenic	Light	Light
Commercial SARS-CoV-2 Detection assays	(EDI™ Novel Coronavirus COVID-19 ELISA Kit)	MAGLUMI IgG/IgM de 2019-nCoV (CLIA)	COVID-19 Ag Respi-Strip

DISCUSSION

Saliva is a reliable tool to detect SARS-CoV-2

Interestingly, saliva can be used as a specimen in molecular

and serological methods aiding in the battle against this life-threatening disease. According to study carried by Azzi et al. salivary samples of COVID-19 patients were successfully analyzed by RT-PCR and the results were compared with their clinical and laboratory data. All the salivary samples were tested positive for the presence of SARS-CoV-2 confirming that saliva is an accurate tool to detect SARS-CoV-2 [43]. Same finding was obtained by a cohort study evaluated by To et al. and they assist the viral load in confirmed COVID patients using salivary swabs [44]. The major advantage of using saliva as a specimen to detect SARS-CoV-2 is the collection method. Compared to nasopharyngeal and throat collection swabs, saliva collection is a non-invasive method which produces comfort to the patients and allow the patients to use home self-sampling techniques. Increasing the active research for the detection of SARS-CoV-2 in the saliva samples is needed as it may provide an accurate and cost-effective test for fast and early detection of the disease [45,46].

CONCLUSION

Testing for SARS-CoV-2 of symptomatic patients is still crucial. Thus, early diagnosis of the disease using an accurate method will help contain the disease and prevent its transmission.

RT-PCR has the superior advantages over the serological method due to its capability to directly detect the viral genetic part without the need for secondary biomarkers like antigens or antibodies. Indeed, the serological based methods are immensely needed for its rapid and ease of use even though the antigen methods still under development and can fail in detection due to the continuously immerging variants. With technological invention, the accuracy and effectiveness of molecular and serological assays will carry on to tackle the SARS-CoV-2 infection. For the future workup, there is a need for collective efforts by researchers to develop portable harmless methods to detect accurately the SARS-CoV-2 variants.

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Conflict of interest

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Informed consent statement

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REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
2. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-269.
3. Kabeerdoss J, Pilania RK, Karkhele R, Kumar TS, Danda D, Singh S. Severe COVID-19, multisystem inflammatory syndrome in children and Kawasaki disease: Immunological mechanisms, clinical manifestations and management. Rheumatol Int. 2021;41(1):19-32.
4. Perlman S, Netland J. Coronaviruses post-SARS: Update on replication and pathogenesis. Nat Rev Microbiol. 2009;7(6):439-450.
5. Aleem A, Ab AS, Slenker AK. Emerging variants of SARS-CoV-2 and novel therapeutics against coronavirus (COVID-19). StatPearls, 2022.
6. Su YY, Zhang SY, Li TD, Xia NS. Early diagnosis and population prevention of coronavirus disease 2019. Curr Opin HIV AIDS. 2020;15(6):345-350.
7. Cheng C, Zhang D, Dang D, Geng J, Zhu P, Yuan M, et al. The incubation period of COVID-19: A global meta-analysis of 53 studies and a Chinese observation study of 11 545 patients. Infect Dis Poverty. 2021;10(05):1-3.
8. Ramchandani R, Kazatchkine M, Liu J, Sudan P, Dybul M, Matsoso P, et al. Vaccines, therapeutics, and diagnostics for covid-19: redesigning systems to improve pandemic response. BMJ. 2021;375.
9. Sharma A, Ahmad Farouk I, Lal SK. COVID-19: A review on the novel coronavirus disease evolution, transmission, detection, control and prevention. Viruses. 2021;13(2):202.
10. Zhou Y, Zhang L, Xie YH, Wu J. Advancements in detection of SARS-CoV-2 infection for confronting COVID-19 pandemics. Lab Invest. 2022;102(1):4-13.
11. Hillen HS, Kokic G, Farnung L, Dienemann C, Tegunov D, Cramer P. Structure of replicating SARS-CoV-2 polymerase. Nature. 2020;584(7819):154-156.
12. Afzal A. Molecular diagnostic technologies for COVID-19: Limitations and challenges. J Adv Res. 2020;26:149-159.
13. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The incubation period of Coronavirus Disease 2019 (COVID-19) from publicly reported confirmed cases: Estimation and application. Ann Intern Med. 2020;172(9):577-582.
14. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045.
15. Caruana G, Croxatto A, Coste AT, Opota O, Lamoth F, Jaton K, et al. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. Clin Microbiol Infect. 2020;26(9):1178-1182.
16. Chen JH, Yip CC, Chan JF, Poon RW, To KK, Chan KH, et al. Clinical performance of the Luminex NxTAG CoV extended panel for SARS-CoV-2 detection in nasopharyngeal specimens from COVID-19 patients in Hong Kong. J Clin Microbiol. 2020;58(8):10-128.
17. Peñarrubia L, Ruiz M, Porco R, Rao SN, Juanola-Falgarona M, Manissero D, et al. Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak. Int J Infect Dis. 2020;97:225-229.
18. de Felice M, de Falco M, Zappi D, Antonacci A, Scognamiglio V. Isothermal amplification-assisted diagnostics for COVID-19. Biosens Bioelectron. 2022;205:114101.

19. Morales-Narváez E, Dincer C. The impact of biosensing in a pandemic outbreak: COVID-19. Biosens Bioelectron. 2020;163:112274.

20. Gaudin M, Desnues C. Hybrid capture-based next generation sequencing and its application to human infectious diseases. Front Microbiol. 2018;9:2924.

21. Mohamadian M, Chiti H, Shoghli A, Biglari S, Parsamanesh N, Esmaeilzadeh A. COVID-19: Virology, biology and novel laboratory diagnosis. J Gene Med. 2021;23(2):e3303.

22. Chen X, Kang Y, Luo J, Pang K, Xu X, Wu J, et al. Next-generation sequencing reveals the progression of COVID-19. Front Cell Infect Microbiol. 2021;11:632490.

23. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.

24. Khalil AM. The genome editing revolution. J Genet Eng Biotechnol. 2020;18(1):1-6.

25. Horodecka K, Düchler M. CRISPR/Cas9: Principle, applications, and delivery through extracellular vesicles. Int J Mol Sci. 2021;22(11):6072.

26. Zuo E, Huo X, Yao X, Hu X, Sun Y, Yin J, et al. CRISPR/Cas9-mediated targeted chromosome elimination. Genome Biol. 2017;18(1):224.

27. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR–Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38(7):870-874.

28. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: Nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019;14(10):2986-3012.

29. Chen JS, Ma E, Harrington LB, da Costa M, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436-439.

30. Akyala AI, Awayimbo JR, Ogo AC, Chima NJ, Billyrose OM, Engom AO. Clinical diagnostic performance evaluation of five immunoassays for antibodies to SARS-CoV-2 diagnosis in a real-life routine care setting. Pan Afr Med J. 2021;39(1):3.

31. Fang B, Meng QH. The laboratory's role in combating COVID-19. Crit Rev Clin Lab Sci. 2020;57(6):400-414.

32. Yu HQ, Sun BQ, Fang ZF, Zhao JC, Liu XY, Li YM, et al. Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. Eur Respir J. 2020;56(2):2001526.

33. Ejazi SA, Ghosh S, Ali N. Antibody detection assays for COVID-19 diagnosis: An early overview. Immunol Cell Biol. 2021;99(1):21-33.

34. Mekonnen D, Mengist HM, Derbie A, Nibret E, Munshea A, He H, et al. Diagnostic accuracy of serological tests and kinetics of severe acute respiratory syndrome coronavirus 2 antibody: A systematic review and meta-analysis. Rev Med Virol. 2021;31(3):e2181.

35. Filchakova O, Dossym D, Ilyas A, Kuanysheva T, Abdizhamil A, Bukasov R. Review of COVID-19 testing and diagnostic methods. Talanta. 2022;244:123409.

36. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel Coronavirus Disease (COVID-19). Clin Infect Dis. 2020;71(15):778-785.

37. Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, et al. Antibody detection and dynamic characteristics in patients with coronavirus disease 2019. Clin Infect Dis. 2020;71(8):1930-1934.

38. Rai P, Kumar BK, Deekshit VK, Karunasagar I, Karunasagar I. Detection technologies and recent developments in the diagnosis of COVID-19 infection. Appl Microbiol Biotechnol. 2021;105:441-455.

39. Mertens P, de Vos N, Martiny D, Jassoy C, Mirazimi A, Cuypers L, et al. Development and potential usefulness of the COVID-19 Ag Respi-Strip diagnostic assay in a pandemic context. Front Med (Lausanne). 2020;7:225.

40. Yüce M, Filiztekin E, Özkaya KG. COVID-19 diagnosis-A review of current methods. Biosens Bioelectron. 2021;172:112752.

41. Stieber F, Howard J, Rao SN, Kawamura LM, Manissero D, Love J, et al. First performance report of QIAreach™ Anti-SARS-CoV-2 total test, an innovative nanoparticle fluorescence digital detection platform. J Clin Virol. 2020;133:104681.

42. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med. 2020;58(7):1081-1088.

43. Azzi L, Carcano G, Gianfagna F, Grossi P, Dalla Gasperina D, Genoni A, et al. Saliva is a reliable tool to detect SARS-CoV-2. J Infect. 2020;81(1):e45-e50.

44. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, 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 Infect Dis. 2020;20(5):565-574.

45. Chiereghin A, Zagari RM, Galli S, Moroni A, Gabrielli L, Venturoli S, et al. Recent advances in the evaluation of serological assays for the diagnosis of SARS-CoV-2 infection and COVID-19. Front Public Health. 2021;8:620222.

46. Gong F, Wei HX, Li Q, Liu L, Li B. Evaluation and comparison of serological methods for COVID-19 diagnosis. Front Mol Biosci. 2021;8:682405.