New COVID-19 saliva-based test: How good is it compared with the current nasopharyngeal or throat swab test?

Kai-Feng Hung[a,b], Yi-Chen Sun[c], Bing-Hong Chen[a], Jeng-Fan Lo[d], Chao-Min Cheng[e], Cho-Yi Chen[f], Cheng-Hsien Wu[b,g,*], Shou-Yen Kao[b,g,*]

[a]Department of Medical Research, Division of Translational Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [b]Department of Dentistry, School of Dentistry, National Yang-Ming University, Taipei, Taiwan, ROC; [c]Department of Ophthalmology, Taipei Tzu Chi Hospital, The Buddhist Tzu Chi Medical Foundation, New Taipei City, Taiwan, ROC; [d]Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan, ROC; [e]Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan, ROC; [f]Institute of Biomedical Informatics, National Yang-Ming University, Taipei, Taiwan, ROC; [g]Department of Stomatology, Taipei Veterans General Hospital, Taipei, Taiwan, ROC

Abstract: As of April 15, 2020, the US Food and Drug Administration has granted emergency use authorization to a first saliva test for diagnosis of severe acute respiratory syndrome coronavirus 2 infection, the device developed by RUCDR Infinite Biologics laboratory, Rutgers University. A key feature that distinguishes the saliva-based test from nasopharyngeal or oropharyngeal (throat) swabs is that this kit allows self-collection and can spare healthcare professionals to be at risk during collecting nasopharyngeal or oropharyngeal samples, thereby preserving personal protective equipment for use in patient care rather than sampling and testing. Consequently, broader testing than the current methods of nasal or throat swabs will significantly increase the number of people screening, leading to more effective control of the spread of COVID-19. Nonetheless, a comparison of saliva-based assay with current swab test is needed to understand what and how we can benefit from this newly developed assay. Therefore, in this mini-review article, we aimed to summarize the current and emerging tools, focusing on diagnostic power of different clinical sampling and specimens.

Keywords: Nasopharynx; Oropharynx; Saliva, Severe acute respiratory syndrome coronavirus 2

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), a respiratory disease that first appeared in China, has spread globally to >200 countries, resulting in over 2.97 million confirmed patients and 206 000 deaths as of April 27, 2020.[1] Since its outbreak last year, research groups used whole genome/RNA sequencing and identified viral cause of COVID-19, which possesses genetic sequence with ~80% similarity to the genome of the severe acute respiratory syndrome virus coronavirus 2 (SARS-CoV).[2–4] The novel coronavirus was hence named SARS-CoV-2. Currently, the most likely transmission route is direct contact and/or air droplet spread,[5,6] which is backed up by the findings that SARS-CoV-2 can be isolated in aerosol (<5 μm) for at least up to 3 hours.[7] Unfortunately, US Food and Drug Administration (FDA) has yet approved any vaccines or therapeutics in clinical use for SARS-CoV-2, and most countries that successfully limit the spread of COVID-19, including Taiwan, primarily rely on rapid case screening, identification, quarantine, and contact tracing. As the symptomatic signs (44%-89% fever, 68% cough, 38% fatigue, 34% sputum production, and 19% short of breath) and computed tomographic scans are non-specific,[8] molecular techniques become the gold standard for COVID-19 diagnosis.[1]

2. CURRENT FIRST-LINE DIAGNOSTIC TEST FOR COVID-19

The reverse transcription polymerase chain reaction (RT-PCR) to detect SARS-CoV-2 is thus far the primary method for diagnosis of COVID-19.[1] The clinical specimens for RT-PCR can be obtained from upper respiratory tract by nasopharyngeal swabs, washes, aspirates, or oropharyngeal swabs, or from lower respiratory tract by sputum collection, bronchoalveolar lavage (BAL), or tracheal aspirates. The specific region serving as the targets for the PCR include the *RdRP* (RNA-dependent RNA polymerase) gene, the *E* (envelop protein) gene, or the *N* (nucleocapsid) gene.[9,10] Meanwhile, the serology tests that examine the production of specific IgM and IgG antibodies against SARS-CoV-2 in response to infection is also useful for surveillance and of value to complement certain limitations of PCR as a sole diagnostic tool. According to US FDA, the SARS-CoV-2 antibodies can

*Address correspondence. Dr. Cheng-Hsien Wu and Dr. Shou-Yen Kao, Department of Stomatology, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: sykao@vghtpe.gov.tw (S.-Y. Kao). (C.-H. Wu)

Author Contributions: Dr. Kai-Feng Hung and Dr. Yi-Chen Sun contributed equally to this work.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2020) 83: 891-894.

Received April 27, 2020; accepted April 27, 2020.

doi: 10.1097/JCMA.0000000000000396.

<zdoi: 10.1097/JCMA.0000000000000396>

be detected several days after initial infection and can still be detectable afterward, thus providing a long period of window for indirectly detecting SARS-CoV-2 for both active and recent past infections.[11] However, as serological assays are currently in development and several challenges remain (such as the cross-reactivity with other virus, as well as the undetermined kinetics of immune response), the RT-PCR still play a pivotal role in the identification of SARS-CoV-2 infection.

3. CLINICALLY RELEVANT ISSUES OF COVID-19 TESTING

Just like the concerns from public health experts for any of the pandemic, two issues of diagnostic testing worth further consideration. In addition to the criteria of who needs to be tested, an important issue relates to the diagnostics itself. Specifically, for RT-PCR, while a positive test result certainly identifies the presence of virus, a negative result may not necessarily rule out SARS-CoV-2 infection. The potential false-negative result could be caused by low virus loads, improper sampling sites and timings, poor technique, and even mutations of viral genome. About the clinical sampling, the US Centers for Disease Control and Prevention (CDC) guideline recommends collecting upper respiratory specimen for asymptomatic patients. For patients who develop a productive cough, sputum can be used for SARS-CoV-2 testing, although the induction of sputum is not recommended. However, nasopharyngeal swab sampling is technically challenging, requires healthcare professionals, and may impose risk for aerosol generation. These drawbacks thus necessitate the implementation of additional diagnostic approach.

4. DESCRIPTION AND PRINCIPLE OF SALIVA-BASED COVID-19 TESTING

The newly approved saliva-based COVID-19 testing kit is built on the existing TaqPath SARS-CoV Assay, developed by the Rutgers Clinical Genomics Laboratory, to qualitatively identify RNA from virus. This assay employs primers and probes validated by the emergency use authorization (EUA) for respiratory, nasopharyngeal, and oropharyngeal specimens. To enable testing saliva specimen, the collection protocols and nucleic acid extraction buffers are modified. Saliva specimens can be transported and stored at ambient temperature but have to be processed within 48 hours of collection. The recommended system for RNA extraction is the PerkinElmer Chemagic 360 with Chemagic Viral DNA/RNA 300 Kit H96. The RT-PCR can be performed using Applied Biosystems TaqPath Combo Kit on the ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System or the Applied Biosystems ViiA7 Real-Time PCR System. The logistics and details can be found at https://www.fda.gov/media/136875/download.

5. COMPARISON OF DIFFERENT TYPES OF CLINICAL SAMPLES AND SPECIMENS FOR SARS-COV-2 DETECTION

As mentioned previously, an accurate identification of respiratory viruses is critically affected by the source of clinical specimens. While several studies on up to 15 common respiratory viruses suggest that the use of nasopharyngeal swabs provides a higher sensitivity than that of nasopharyngeal washings or oropharyngeal swabs,[12,13] this is not necessarily the case for SARS-CoV-2, as the infectivity and the predilection for transmission may differ significantly between viruses. In addition, even if a given type of clinical specimen offers a relatively higher accuracy in diagnosis, it remains an open question whether the technique-demanding test is the most needed during a pandemic with global shortage of medical supplies as of today.

Currently, the available data comparing the sensitivity for SARS-CoV-2 detection using nasal, pharyngeal, or oral swab are very limited. One study from a Chinese group examined 213 hospitalized SARS-CoV-2 patients with a total of 205 oropharyngeal and 490 nasopharyngeal swabs at various time points of disease course. They found that nasopharyngeal swabs have overall higher positive rates (53.6%-73.3%) than oropharyngeal (throat) swabs (11.1%-61.3%), regardless of whether the patients were in mild or severe disease conditions. Notably, this study showed highest positive rate using sputum specimens, which is generally regarded as a type of lower respiratory tract sample.[14] Separately, a study examining the sensitivity of SARS-CoV-2 detection with different clinical samples from 205 patients in China showed that BAL fluid has highest positive rate (93%), followed by sputum (72%), nasal swabs (63%), brush biopsy (46%), and pharyngeal swabs (32%).[15] In contrast to the findings from these studies, another study examining nine hospitalized COVID-19 patients in Germany showed that there are no discernible differences in virus loads or positive rates between nasopharyngeal versus oropharyngeal swabs, with an overall detection rate of 45.95% being reported, although the numbers of nasopharyngeal and oropharyngeal swabs taken were not described. Notably, this study found that only two among nine patients have higher virus load (>3 in threshold cycle [Ct] value) in sputum samples than swabs, thus leading to the conclusion that simple throat swabs will provide sufficient sensitivity for screening.[16]

In addition to nasopharyngeal and oropharyngeal swabs, a few groups also examined the potential of saliva as the clinical specimen for SARS-CoV-2 detection. In this regard, a study of 12 patients confirmed by PCR-detection of virus RNA using nasopharyngeal or sputum specimens found that the coughed-out saliva from 11 patients were positive for SARS-CoV-2.[17] Importantly, virus RNA was not detected in saliva samples collected from another 33 patients whose nasopharyngeal specimens were tested negative for SARS-CoV-2. Consistent results were obtained by the same group examining a different set of patients, showing that SARS-CoV-2 was detectable in self-collected saliva of 20 of 23 confirmed patients.[17] These studies revealed that salivary virus loads corresponded to the severity of disease and declined after treatment, although the differences were not statistically significant possibly because of the small sample size. Similar to those studies with nasal or throat samples, these reports showed that the SARS-CoV-2 can still be detected in saliva among a third of patients 20 days or longer after initial diagnosis, thus supporting the idea that saliva may represent as an appropriate specimen for screening patients ever with SARS-CoV-2 infection.

Another approach to collect saliva sample is mediated by oral swabs, which is easily applicable even for non-professional individuals. In two studies examining saliva sample collected by oral swabs, 15 out of 39 (50%) and 25 out of 25 (100%) patients were tested SARS-CoV-2 positive, respectively.[18,19] Although it remains premature to reach any conclusion, these studies indeed imply that the saliva, either collected by coughing out or oral swabs, is a legitimate clinical specimen for SARS-CoV-2 detection.

6. SALIVA PROVEN TO BE A VALUABLE CLINICAL SPECIMEN FOR DETECTION OF SARS-COV: LESSONS FROM 17 YEARS AGO

As both of the COVID-19 and SARS are caused by coronaviruses and can be transmitted through respiratory droplets, the studies on SARS-CoV may provide a hint as we continue to navigate

and unravel COVID-19. Concerning the value of saliva as the clinical specimen for coronavirus detection, a study of 17 SARS cases in Taiwan showed that a substantial amount of SARS-CoV RNA were detected in saliva (7.1×10^3 to 6.4×10^8 copies/mL) and throat wash (9.6×10^2 to 5.9×10^6 copies/mL) from all patients. Importantly, the highest detection rate of saliva/throat wash samples appeared as early as 4 days after disease onset, thus implying that these clinical specimens can be used for virus detection.[20] Another previous study examined the SARS-CoV loads in different clinical samples and found that the virus RNA could be detected in saliva (5.2×10^2 copies/mL), although its level was relatively lower than that in throat swabs (5.5×10^2 copies/mL), sputum (1.2×10^6 copies/mL), and endotracheal aspirates (2.8×10^6 copies/mL).[21] It is noted that the amounts of SARS-CoV virus RNA detected in these two studies differ by a significant amount, which can be possibly associated with the timing of sampling: The samples in the first study were taken between day 2 and day 9, whereas the samples in the second study were taken after a median duration of 12 days (2 to 54 days) after the onset of symptoms. Indeed, the observation that SARS-CoV and other coronavirus peaks at around 10 days after onset of disease was commonly shared between studies of various clinical samples.[22–24] Collectively, saliva is of potential diagnostic value for and should play a role in detection of SARS-CoV-2 infection.

7. GENERAL CONSIDERATION TO USE SALIVA AS A DIAGNOSTIC FLUID FOR VIRUS DETECTION

Since saliva is easily collected and clinically informative for disease detection, the consideration that maximizes the benefit of using saliva as a diagnostic fluid deserves more attention. Thus far, the approach and protocol for collection of saliva sample has yet officially standardized; however, it is likely that the diagnostic value of saliva is closely related to how saliva sample is obtained. This concept is supported by a study examining saliva specimen collected directly from the opening of salivary glands of 31 confirmed cases, showing that only four patients (12.9%) were tested positive for SARS-CoV-2 detection,[25] which is significantly lower than the positive rate derived from examination of coughed-out saliva. It is possible that the advantage of using the spit saliva is partly attributed to the potential availability for multiple targets, such as desquamated oropharyngeal mucous epithelial cells and respiratory secretions with shedding viruses. This concept is supported by a previous study, which found replicating SARS-CoV in the cells collected by throat wash from SARS patients. This characteristic of benefit stands for the sputum and saliva. Indeed, it has been estimated that the SARS-CoV-2 load of sputum is 10^6 to 10^{11} particles/mL, whereas the virus load of saliva is 10^8 to 10^9 particles/mL;[26–28] however, unlike the sputum comprising a large amount of mucus that hampers RNA extraction, saliva (~70% to 90% water) is supposed to give at least a comparable load of viral RNA. As to the sampling protocols, a 0.5-hour or up to overnight fasting before saliva collection has been shown in multiple studies to increase the concentration of RNA.[29–32] It is also recommended to have the subject rinse their mouth with water but not disinfectant mouthwash. The same guidelines should be used for both of the spitting/coughing out and oral swab approaches.

In conclusion, the diagnostic testing is crucial for controlling the COVID-19 pandemic. Any implementation of clinical sampling for diagnosis should take into considerations of the sensitivity of assays, risks to healthcare professionals, and global shortage of equipment. Many studies showed that sputum is superior to nasopharyngeal swabs in detection of SARS-CoV-2 infection. However, while the virus is often reliably detected in sputum, this clinical specimen is not always obtainable for patients without productive coughs and induction of cough may even enhance the spread of virus. On the other hand, several preliminary reports showed that the viral load in saliva is comparable with that in sputum. Moreover, the collection of saliva is minimally invasive and can be self-administrated. Accordingly, the saliva-based SARS-CoV-2 diagnostics seems to be potentially promising and appealing. Notably, this is a rapidly moving research topic and the current evidence is not peer-reviewed and, therefore, is still far from leading to a solid conclusion. Nevertheless, it is reasonable to incorporate the saliva-based SARS-CoV-2 assay into a part of multiple lines of diagnostics, which believably may further facilitate the identification of COVID-19 patients.

REFERENCES

1. Centers for Disease Control and Prevention. Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19). 2020.
2. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W 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; 200642. Doi: 10.1148/radiol.2020200642.
3. 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**:270–3.
4. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565-74.
5. Centers for Disease Control and Prevention. How COVID-19 Spreads. 2020.
6. Li X, Zai J, Wang X, Li Y. Potential of large "first generation" human-to-human transmission of 2019-nCoV. *J Med Virol* 2020;**92**:448–54.
7. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020;**382**:1564–7.
8. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020. Doi:10.1056/NEJMoa2002032.
9. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe* 2020;**27**:325–8.
10. Sexton NR, Smith EC, Blanc H, Vignuzzi M, Peersen OB, Denison MR. Homology-based identification of a mutation in the coronavirus RNA-dependent RNA polymerase that confers resistance to multiple mutagens. *J Virol* 2016;**90**:7415–28.
11. Haveri A, Smura T, Kuivanen S, Osterlund P, Hepojoki J, Ikonen N et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. *Euro Surveill* 2020; **25**. Doi: 10.2807/1560-7917.ES.2020.25.11.2000266
12. Lieberman D, Lieberman D, Shimoni A, Keren-Naus A, Steinberg R, Shemer-Avni Y. Identification of respiratory viruses in adults: nasopharyngeal versus oropharyngeal sampling. *J Clin Microbiol* 2009;**47**:3439–43.
13. Li L, Chen QY, Li YY, Wang YF, Yang ZF, Zhong NS. Comparison among nasopharyngeal swab, nasal wash, and oropharyngeal swab for respiratory virus detection in adults with acute pharyngitis. *BMC Infect Dis* 2013;**13**:281.
14. Yang YYM, Shen C, Wang F, Yuan J, Li J, et al Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. *medRxiv* 2020; 02.11.20021493.
15. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020. Doi:10.1001/jama.2020.3786.
16. Roman Wölfel VMC, Wolfgang G, Michael S, Sabine Z, Marcel A. Müller D et al. Virological assessment of hospitalized cases of coronavirus disease 2019. *medRxiv* 2020. https://doi.org/10.1038/s41586-020-2196-x.
17. To KK, Tsang OT, Chik-Yan YC, Chan KH, Wu TC, Chan JMC, et al. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 2020. Doi:10.1093/cid/ciaa149.

18. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina D, Genoni A, et al. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 2020. Doi:10.1016/j.jinf.2020.04.005.
19. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 2020;**9**:386–9.
20. Wang WK, Chen SY, Liu IJ, Chen YC, Chen HL, Yang CF, et al. SARS Research Group of the National Taiwan University/National Taiwan University Hospital. Detection of SARS-associated coronavirus in throat wash and saliva in early diagnosis. *Emerg Infect Dis* 2004;**10**:1213–9.
21. Drosten C, Chiu LL, Panning M, Leong HN, Preiser W, Tam JS, et al. Evaluation of advanced reverse transcription-PCR assays and an alternative PCR target region for detection of severe acute respiratory syndrome-associated coronavirus. *J Clin Microbiol* 2004;**42**:2043–7.
22. Oh MD, Park WB, Choe PG, Choi SJ, Kim JI, Chae J, et al. Viral load kinetics of MERS coronavirus infection. *N Engl J Med* 2016;**375**:1303–5.
23. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat Rev Microbiol* 2009;**7**:439–50.
24. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. HKU/UCH SARS Study Group. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 2003;**361**:1767–72.
25. Chen L. Detection of 2019-nCoV in saliva and characterization of oral symptoms in COVID-19 patients. *Lancet* 2020. Doi:10.2139/ssrn.3556665.
26. Roman C, Wolfgang G, Michael S, Sabine Z, Marcel AM, Daniela N, et al. Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019 in a travel-associated transmission cluster. *medRxiv* 2020. Doi:10.1101/2020.3.05.20030502.
27. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 2020;**20**:411–2.
28. Yinon F, Rob P, Ron M. SARS-CoV-2 (COVID-19) by the numbers. *medRxiv* 2020. Doi:10.7554/eLife.57309.
29. Ishikawa S, Sugimoto M, Kitabatake K, Sugano A, Nakamura M, Kaneko M, et al. Identification of salivary metabolomic biomarkers for oral cancer screening. *Sci Rep* 2016;**6**:31520.
30. Goode MR, Cheong SY, Li N, Ray WC, Bartlett CW. Collection and extraction of saliva DNA for next generation sequencing. *J Vis Exp* 2014; **4**:253.
31. Laidi F, Bouziane A, Lakhdar A, Khabouze S, Amrani M, Rhrab B, et al. Significant correlation between salivary and serum Ca 15-3 in healthy women and breast cancer patients. *Asian Pac J Cancer Prev* 2014;**15**: 4659–62.
32. Mandel ID. The diagnostic uses of saliva. *J Oral Pathol Med* 1990;**19**:119–25.