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SHORT COMMUNICATION

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Deep throat saliva as an alternative diagnostic specimen type for the detection of SARS-CoV-2

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Abstract

Nasopharyngeal swabs (NPS) are widely accepted as specimens for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the current pandemic of coronavirus disease 2019. However, the collection procedures for NPS specimens causes sneezing and coughing in most patients, which generate droplets or aerosol particles that are hazardous to the healthcare workers collecting these specimens. In this study, 95 patient-matched paired deep throat saliva (DTS) and NPS specimens from 62 patients were analyzed. Samples were tested for SARS-CoV-2 by reverse-transcription polymerase chain reaction (RT-PCR). The rates of detection for DTS (53.7%) and NPS (47.4%) samples were comparable ($P = .13$). It is important to note that the patients should be clearly instructed or supervised during DTS collection. In conclusion, SARS-CoV-2 detection by RT-PCR was equivalent in DTS and NPS specimens.

KEYWORDS

COVID-19, deep throat saliva, RT-PCR, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) refers to a cluster of viral pneumonia cases that were first identified in December 2019 in Wuhan, a city in the Hubei Province of China.[1] A novel coronavirus, currently known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the causative agent of COVID-19. Subsequently, COVID-19 has spread across the world to 214 countries in Asia, North America, South America, Europe, Australia and Africa, affecting 3 267 184 people, including 229 971 deaths thus far.[2] Health authorities worldwide are seeking effective public-health interventions for fighting against this pandemic. One way to effectively manage the COVID-19 pandemic is to enhance laboratory testing and obtaining the appropriate sample type is a key component of accurate diagnostic testing. Real-time reverse transcription-polymerase chain reaction (RT-PCR) of nasopharyngeal and lower respiratory specimens is widely used to detect SARS-CoV-2.[3,4] However, collecting nasopharyngeal specimens causes patient discomfort and may trigger sneezing and coughing, which could generate droplets or aerosol particles that are hazardous to the healthcare workers collecting the samples.[5,6] Therefore, saliva has been proposed as an alternative specimen type for the diagnosis of COVID-19. As saliva could be collected by the patients themselves, it could reduce the nosocomial transmission risk to healthcare workers.[7,8] Furthermore, the use of saliva could reduce the demand for swabs, which is currently in short supply.

The performance of saliva as a diagnostic specimen type for the detection of respiratory viruses and SARS-CoV-2 has been previously studied.[9,10] However, these studies lacked detail on the mode of specimen collection, in particular, whether deep throat (posterior oropharyngeal) saliva and not oral saliva, should be recommended for use as a type of diagnostic specimen. This study aimed to assess the comparability of deep throat saliva (DTS) samples to nasopharyngeal swab (NPS) samples as an alternative specimen type for the detection of SARS-CoV-2 by RT-PCR.

1.1 | Study design

A retrospective study was conducted from February to March 2020, analyzing patient-matched paired specimens of DTS and NPS collected on the same day from patients admitted to the Prince of Wales

Hospital in Hong Kong. To collect DTS samples, patients were provided clear instructions to collect saliva from the deep throat (posterior oropharyngeal) in a sterile sputum container.[8] In-house prepared viral transport medium (2 mL) was added in the laboratory for sample processing. NPS samples were collected by the nursing staff using flocked swabs in a container with 3 ml viral transport medium. Collected specimens were sent to the laboratory immediately for SARS-CoV-2 RT-PCR testing.

1.2 | Reverse transcription-polymerase chain reaction assay

An RT-PCR assay using the lightMix Modular SARS-CoV (COVID19) E-gene detection kit (TIB Molbiol, Berlin, Germany), which target a 78 base pair fragment from a conserved region in the E gene of SARS CoV-1, SARS CoV-2, and the bat-associated SARS-related virus (Sarbecovirus), was used as a screening assay. Briefly, nucleic acid extraction was performed using the MagMAX (Applied Biosystems, Foster city) and a viral RNA isolation kit (Applied Biosystems), and 50 µL of viral RNA was obtained from every 200 µL sample. A 20 µL reaction mix containing 5 µL of 4X TaqMan fast (Applied Biosystems), 0.5 µL primer-probe mix, 4.5 µL Nuclease-free water, and 10 µL nucleic acid was prepared. RT-PCR was conducted using the ABI 7900 real-time PCR system (Applied Biosystems) under the following conditions: 5 minutes at 55°C, 20 seconds at 95°C, 40 cycles of 3 seconds at 95°C, and 30 seconds at 60°C. The samples that tested positive for SARS-CoV-2 were sent to the Public Health Laboratory Service Branch in Hong Kong for confirmation, where they used a different RT-PCR assay that targeted a SARS-CoV-2 specific RdRp gene region.

1.3 | Data analysis

The SARS-CoV-2 detection rates for DTS and NPS were compared by Pearson's χ^2 test. Analysis of correlation agreement methods, such as percent agreement and κ statistic, were used to determine the comparability of the two sampling methods. Statistical analyses were performed using the MedCalc statistical software version 16.4.3 (Ostend, Belgium).

2 | RESULTS

This study analyzed 95 patient-matched paired samples from 62 patients including 29 confirmed patients with COVID-19 and 33 COVID-19 negative patients, of which 26 were males aged 19 to 85 years (mean age: 42.0 ± 17.1 years). The comparison of clinical performance between DTS and NPS samples, based on the RT-PCR detection of SARS-CoV-2, are shown in Table 1. There were no statistical differences between the detection rates of DTS and NPS ($P > .05$). With regard to the correlation, the overall agreement between the two sampling methods was 78.9% and the kappa value was 0.58, indicating moderate agreement between these two sample types.

TABLE 1 Clinical performance comparison between DTS and NPS for detecting SARS-CoV-2

	Paired samples (n = 95)	
	DTS[a]	NPS[b]
No. of RT-PCR positive sample (%)	51 (53.7)	45 (47.4)
No. of RT-PCR negative sample (%)	44 (46.3)	50 (52.6)
Overall agreement (95% CI[c])	78.9% (69.1%-86.4%)	
Kappa (95% CI)	0.58 (0.42-0.74)	
P[d]	0.13	

Abbreviations: CI, confidence interval; DTS, deep throat saliva; NPS, nasopharyngeal swabs; RT-PCR, reverse transcription-polymerase chain reaction.
[a]Deep throat saliva.
[b]Nasopharyngeal swab.
[c]Confidence internal.
[d]P value from Pearson's χ^2 comparison.

There were 75 concordant samples and 20 discordant samples between the two specimen types evaluated. The assessment of SARS-CoV-2 RT-PCR results of DTS and NPS samples from confirmed patients with COVID-19 are listed in Table 2. The 20 discordant samples pair were collected from 12 confirmed patients with COVID-19, of which 13 DTS samples that showed positive results had corresponding NPS samples that were negative. Seven NPS samples that showed positive results had corresponding negative DTS samples. When evaluating the cycle threshold (C_t) values obtained from the positive results, the discordant pairs of negative NPS/positive DTS samples had positive C_t values ranging from 33.7 to 37.9, whereas the discordant pairs of negative DTS/positive NPS samples had values that ranged from 23.9 to 35.9. In general, DTS samples had a higher positive detection rate than NPS samples, although there were no statistically significant differences between the two sampling methods ($P > .05$). All negative samples from the discordant pairs could be considered as low viral load positive samples, except sample 70 from patient AY, which exhibited negative DTS and positive NPS results (C_t value 23.9).

3 | DISCUSSION

Nasopharyngeal specimens, either from nasopharyngeal aspirates (NPA) or NPS, are the recommended specimen type for the detection of respiratory viruses.[11] The NPS is also the most common validated specimen type for a majority of the commercially available respiratory virus detection kits. Thus, NPA or NPS is also the recommended specimen type for SARS-CoV-2 detection. Given the invasive nature of the procedures used to obtain NPA, NPS specimens were the specimen of choice for SARS-CoV-2 detection. The collection of NPS, although less invasive than NPA, may cause

TABLE 2 The assessment of SARS-CoV-2 RT-PCR results of NPS and DTS samples pairs from 29 confirmed patients with COVID-19 (n = 61)

Patient	Sample pair	SARS-CoV-2 RT-PCR (C_t[a] value) NPS[b]	DTS[c]	Assessment
AE	20	P (21.3)	P (25.4)	Concordant
	21	P (25.9)	P (31.3)	Concordant
	22	P (29.3)	P (33.2)	Concordant
	23	P (32.4)	P (33.0)	Concordant
	24	P (33.3)	P (34.0)	Concordant
	25	N (0)	P (36.7)	Discordant
AB	8	P (22.3)	P (19.9)	Concordant
	9	P (28.8)	P (24.6)	Concordant
	10	P (28.4)	P (27.7)	Concordant
	11	P (27.7)	P (25.2)	Concordant
	12	P (33.1)	N (0)	Discordant
	13	N (0)	P (35.8)	Discordant
AJ	34	P (29.6)	P (23.5)	Concordant
	35	N (0)	P (36.4)	Discordant
	36	N (0)	P (36.0)	Discordant
	37	N (0)	N (0)	Concordant
BC	77	P (27.6)	P (31.5)	Concordant
	78	P (34.6)	N (0)	Discordant
	79	N (0)	P (36.1)	Discordant
	80	N (0)	P (37.9)	Discordant
AN	45	P (35.6)	P (32.4)	Concordant
	46	N (0)	P (35.3)	Discordant
	47	P (33.9)	N (0)	Discordant
BB	75	N (0)	P (33.7)	Discordant
	76	P (34.5)	N (0)	Discordant
AY	70	P (23.9)	N (0)	Discordant
	71	P (30.6)	N (0)	Discordant
AV	59	N (0)	P (35.4)	Discordant
	60	N (0)	P (36.0)	Discordant
BA	73	P (21.2)	P (30.8)	Concordant
	74	P (35.9)	N (0)	Discordant
AC	14	N (0)	P (37.6)	Discordant
AO	48	N (0)	P (33.8)	Discordant
AZ	72	N (0)	P (35.6)	Discordant
AI	30	P (32.8)	P (34.5)	Concordant
	31	P (36.4)	P (32.1)	Concordant
	32	N (0)	N (0)	Concordant
	33	P (37.4)	P (33.5)	Concordant
BH	90	P (22.1)	P (27.9)	Concordant
	91	P (28.4)	P (32.2)	Concordant
	92	P (33.9)	P (35.4)	Concordant
AX	67	P (22.7)	P (27.0)	Concordant
	68	P (31.7)	P (30.1)	Concordant
AW	65	P (19.8)	P (32.2)	Concordant
	66	N (0)	N (0)	Concordant
AP	51	P (19.7)	P (22.3)	Concordant
	52	P (25.7)	P (25.7)	Concordant
AR	54	P (17.4)	P (20.0)	Concordant
	55	P (29.6)	P (32.2)	Concordant
BE	82	P (32.6)	P (32.5)	Concordant
	83	P (34.5)	P (35.9)	Concordant
AH	28	P (24.1)	P (22.6)	Concordant
AS	56	P (18.2)	P (22.3)	Concordant
AT	57	P (23.9)	P (21.2)	Concordant
AU	58	P (34.3)	P (34.0)	Concordant
AM	42	P (27.9)	P (30.2)	Concordant
AF	26	P (17.0)	P (21.3)	Concordant
AA	1	P (32.0)	P (25.9)	Concordant
BG	89	P (19.6)	P (22.7)	Concordant
AQ	53	P (26.5)	P (30.6)	Concordant
BF	84	P (25.2)	P (31.0)	Concordant

Abbreviations: COVID-19, coronavirus disease 2019; DTS, deep throat saliva; NPS, Nasopharyngeal swabs; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV2, severe acute respiratory syndrome coronavirus 2.

[a]Cycle threshold.

[b]Nasopharyngeal swab.

[c]Deep throat saliva.

patients to cough, thus increasing the risk to healthcare workers. To combat the COVID-19 pandemic, it is crucial to increase laboratory testing for SARS-CoV-2. However, dramatically increasing NPS collection would increase the nosocomial transmission risk to healthcare workers who undertake the specimen collection. In this study, the potential of patient self-collected DTS as an alternative specimen type for the detection of SARS-CoV-2 was assessed.

This study showed that the overall detection rate for SARS-CoV-2 from DTS was comparable to that from NPS samples. The detection rate of DTS samples (53.7%) was even higher than NPS samples (47.4), although the difference was not statistically significant ($P > .05$). This finding is especially critical at the moment, because there is a demand for alternative diagnostic specimen, such as DTS, for SARS-CoV-2 detection. DTS specimens can be easily provided by the patient and do not require healthcare workers for their collection. Furthermore, because of the sudden increase in the number of laboratory tests, DTS provides an alternative that could alleviate the global shortages for swabs and personal protective equipment.

A total of 20 discordant paired samples from 12 confirmed patients with COVID-19 were found in this study. By evaluating the C_t value of these samples, all but one (sample 70: NPS positive C_t value 23.9/negative DTS) had low viral loads (high C_t value). The lone outlying pair may be explained by a discrepancy in the quality of the NPS and DTS samples, which differed in their collection methods, since the NPS samples were collected by trained healthcare personnel, while DTS samples were collected by the patients themselves. When the suitability of DTS for testing was evaluated, it was observed that the quality of samples could be dependent on the patient's understanding of the collection procedure for deep throat (posterior oropharyngeal) saliva. Thus a pre-collection briefing and explanation of this procedure are very important. Based on past experience, clear instructions with color-printed step-by-step procedures or video demonstration can help patients understand the collection procedures. In addition to clear patient instructions, it is also recommended that a nurse should be present to supervise during the collection process.

According to reports, the Hong Kong health department has been using DTS for the detection of SARS-CoV-2 in asymptomatic returnees at the airport. By using DTS samples, mass screening could be implemented as the returnees are required to collect their own specimens and thus, could also prevent crowding at the sample collection centers. Currently, DTS is not a validated sample type for commercially available detection kits of SARS-CoV-2 or other respiratory viruses. To enhance the flexibility of laboratory testing strategies, vendors should include DTS as an alternative validated specimen type.

There are several limitations to this study. First, this is a single-center study. Second, the sample size was small and only 95 paired DTS and NPS samples were selected for comparison. Third, the DTS and NPS samples were not collected at the same time, but on the same day.

4 | CONCLUSION

This study showed that the overall performance of DTS was equivalent to that of the NPS, as specimens used in the detection of SARS-CoV-2 by RT-PCR assay.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBTUONS

Dr. Eddie Leung carried out the experiments, performed the analysis and drafted the manuscript. Dr. Viola Chow and Dr. May Lee contributed to the interpretation of the results. Dr. Raymond Lai conceptualized and supervised the project. All authors discussed the results and contributed to the final manuscript.

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REFERENCES

1. World Health Organisation. Novel Coronavirus (2019-nCoV). Situation report-1. 2020.
2. World Health Organisation. Coronavirus disease (COVID-19). Situation report-103. 2020.
3. Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *J Med Virol*. 2020;92(6):538-539.
4. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med*. 2020;382(12):1177-1179.
5. Frazee BW, Rodríguez-Hoces de la Guardia A, Alter H, et al. Accuracy and discomfort of different types of intranasal specimen collection methods for molecular influenza testing in emergency department patients. *Ann Emerg Med*. 2018;71(4):509-517.
6. Centers for Disease Control and Prevention. Influenza specimen collection reference guide. https://www.cdc.gov/flu/pdf/professionals/flu-specimen-collection-poster.pdf. Accessed at 16 June 2020.
7. To KKW, Yip CCY, Lai CYW, et al. Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. *Clin Microbiol Infect*. 2019;25(3):372-378.
8. 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 Infect Dis*. 2020;20(5):565-574.
9. Azzi L, Carcano G, Gianfagna F, et al. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect*. 2020;81(1):45-50.
10. To KKW, Tsang OTY, Yip CCY, et al. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis*. 2020:149.
11. World Health Organisation. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases, Interim guidance. 2020.

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