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Verification of the efficiency of saline gargle sampling for detection of the Omicron variant of SARS-CoV-2, a pilot study

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SUMMARY A saline gargle (SG) has proven to be an efficient method of sampling to detect SARS-CoV-2. The aim of this pilot study was to verify the efficiency of SG sampling in detecting the Omicron variant of SARS-CoV-2. Subjects were a total of 68 patients with COVID-19 (Omicron variant), and 167 pairs of samples were collected. A conventional oropharyngeal swab (OPS) was obtained and SG sampling was performed immediately afterward; both were subjected to RT-qPCR. A subgroup analysis of symptomatic and asymptomatic patients was performed. Results revealed no significant differences in the distribution of patients and cycle threshold (CT) values between the SG and OPS in overall data and data on days 1-3, 4-7, and 8-14. The subgroup analysis revealed no significant differences between the SG and OPS results in symptomatic patients. In asymptomatic patients, the CT values for the SG were significantly lower than those for the OPS, implying that SG sampling had better sensitivity in the context of the Omicron variant. These data indicate that the SG had satisfactory efficiency (*vs*. the OPS). An SG is a simple and less invasive method of sampling that is suited to mass, frequent, and repeated sampling to detect SARS-CoV-2.

Keywords saline gargle, SARS-CoV-2 detection, Omicron variant, oropharyngeal swab, COVID-19

By far, the gold standard for confirming COVID-19 remains SARS-CoV-2-specfic quantitative real-time polymerase chain reaction (RT-qPCR) sampling with a nasopharyngeal swab (NPS) (*1*). An NPS has the best sensitivity, but it can cause discomfort. Moreover, sampling with an NPS commonly requires well-trained and experienced medical staff. More convenient methods, such as an oropharyngeal swab (OPS), are also widely accepted for mass, frequent, and repeated sampling, but the latter sacrifices convenience and sensitivity. In China, an OPS is now the most widely used method of sampling to detect SARS-CoV-2. However, an OPS can also cause discomfort to the examinee. It can potentially enhance the exposure risk of medical staff since an OPS can sometimes cause coughing. Accordingly, alternative methods of sampling that are less invasive have been considered. Saliva testing is an important alternative method that is also accepted in many countries such as Japan. However, saliva sampling remains controversial because of its sensitivity (*vs*. an NPS and OPS). Moreover, a lack of standard methods of collection/processing has also been cited by some researchers (*2*), which consider saliva sampling inappropriate for the general population. Tan *et al*. pointed out that standardization of saliva sampling might be a solution to encourage it acceptance as an alternative method of detecting SARS-CoV-2 (*3*). However, developing a "replicable" standard method of saliva sampling is not easy. Accordingly, an alternative method of sampling should have the following characteristics: safe, convenient, sensitive, comfortable, simple, and replicable, so that it can easily be standardized. Thus, a saline gargle (SG) has been considered. Bennett *et al*. found that a gargle lavage sample is more sensitive than an OPS for respiratory pathogens (*4*). Early in 2020, Saito *et al*. first reported that testing a gargle sample for SARS-CoV-2 using 10 mL of normal saline can yield a positive result from a patient with COVID-19. They pointed out that an SG might be used as a safe and sensitive method with which to diagnose COVID-19 (*5*). Mittal *et al*. compared the efficiency of SARS-CoV-2 detection among an NPS, OPS, and SG (8-10 mL of normal saline) (*6*). They

found that an SG had satisfactory sensitivity at detecting SARS-CoV-2 but caused less discomfort. Later, Poukka *et al*. (*7*) and Lévesque *et al*. (*8*) also obtained similar results. Benoit *et al*. found that gargling with water for 5 s in the mouth and 5 s in the throat had a slightly lower sensitivity *vs*. an OPS and NPS (*9*). Gobeille Paré *et al*. found that gargling with natural spring water (5 mL of water for 20s) resulted in a lower sensitivity than an OPS or NPS (89.6% *vs*. 97.9%, $p = 0.005$) (*10*). Most of the aforementioned studies demonstrated the value of an SG as an alternative method of sampling to detect SARS-CoV-2, though it is less sensitive than conventional OPS and NPS. However, these studies are based on the old variants such as the beta and delta strains. Thus far, no study has verified the diagnostic value of an SG in detecting the Omicron variant, which mainly affects the upper respiratory tract. Moreover, most patients are asymptomatic. Given these facts, the current study was designed to evaluate the sensitivity of an SG in the context of the Omicron variant. A subgroup analysis was performed by dividing subjects into symptomatic and asymptomatic patients. This study attempted to determine the value of an SG in symptomatic and asymptomatic patients. The findings of this study may help to better understand the role of an SG in SARS-CoV-2 detection.

Subjects were a total of 68 patients who were confirmed to be infected with the Omicron variant of SARS-CoV-2 (2022 July-2022 August) based on an NPS. The inclusion and exclusion criteria are available in the supplementary materials (Table S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=128*). Subjects were tested daily (9:00 AM) for SARS-CoV-2 with an OPS and an SG immediately afterwards after the initiation of this study. A total of 167 pairs of samples (including OPSs and SGs) were collected. The OPS was obtained per routine methods. SG samples were collected by asking subjects to rinse their mouth with 8 mL of saline water for 10 seconds. They then tilted their head back and gargled for another 10 seconds, finally spitting the water back into a 10-mL plastic tube. Samples were collected and stored in a refrigerator at -80℃. The time between sampling and refrigeration was limited to 4 hours. This study was strictly conducted per the guidelines of the Declaration of Helsinki of the World Medical Association (2000), and it was approved and supervised by the ethics committee of The Third People's Hospital of Shenzhen (approval number 2022-116-03). This study was registered with a Chinese clinical trial registry (ChiCTR2200063457). The study protocol was explained to all of the patients, who were asked to provide written informed consent to participation in this study. Samples were treated and tested for SARS-CoV-2 using a routine RT-qPCR assay. The software SPSS (ver23.00, IBM, US) was used for statistical analysis. Categorical variables were expressed as a percentage while continuous variables were expressed as a median with an interquartile range (IQR). The distribution of positive and negative patients was compared using a chi-squared test or Fisher's exact test. A paired *t*-test was used to compare the difference in the CT values for the SG and OPS (CT values were only available for positive patients). The detailed methodology is available in the supplementary materials.

This study involved a total of 68 subjects with COVID-19 (Omicron variant). Detailed information on the patients is listed in Table S2 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=128*). Overall, a total of 126 (77.45%) SG samples tested positive, 12 (7.19%) of those had a single positive result, and 41 tested negative. Overall, a total of 126 (77.45%) OPS samples tested positive, and 11 (6.59%) of those had a single positive result. There were no significant differences in the distribution of

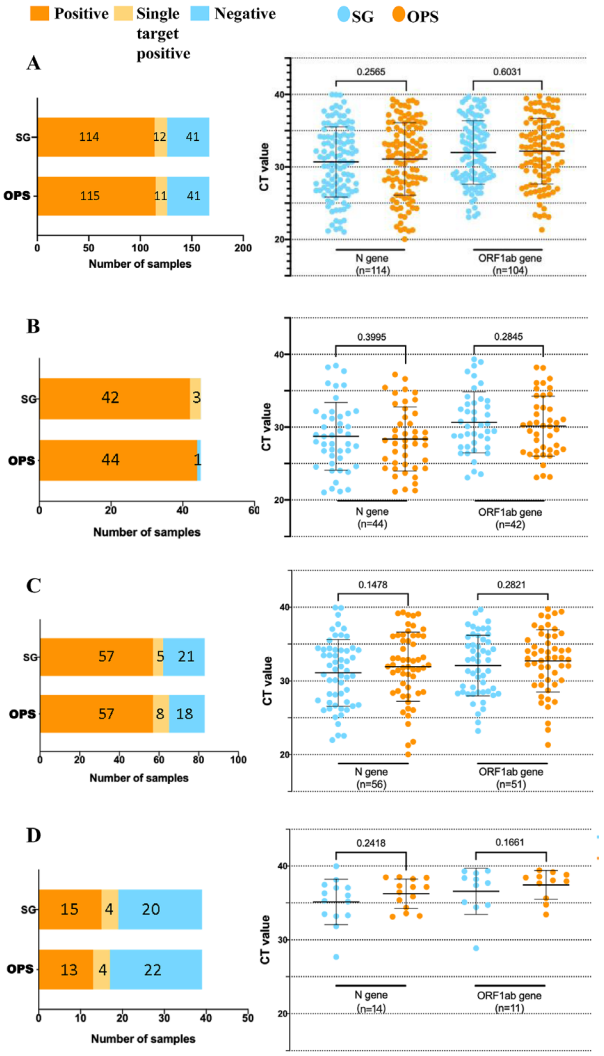

Figure 1. Comparison of SARS-CoV-2 detection in a saline gargle or oropharyngeal swab from subjects infected with the Omicron variant of SARS-CoV-2. (A), Overall data on SARS-CoV-2 detection in all patients. **(B)**, Data 1-3 days after the onset of COVID-19. **(C)**, Data 4-7 days after the onset of COVID-19. **(D)**, Data 8-14 days after the onset of COVID-19. The column on the left indicates the distribution of patients tested with RT-qPCR; the column on the right indicates the CT values determined with RT-qPCR. CT, cycle threshold; OPS, oropharyngeal swab; SG, saline gargle.

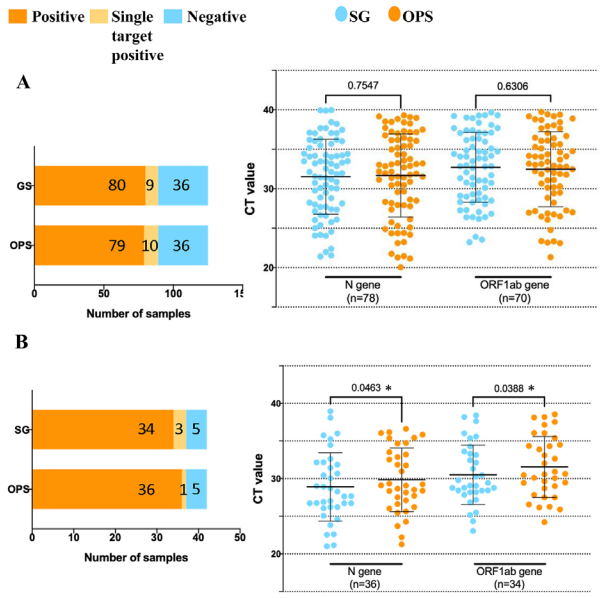

Figure 2. Comparison of SARS-CoV-2 detection (Omicron variant) in a saline gargle or oropharyngeal swab from symptomatic or asymptomatic subjects with COVID-19. (A), Data on symptomatic patients. **(B)**, Data on asymptomatic patients. The column on the left indicates the distribution of patients tested with RT-qPCR; the column on the right indicates the CT values determined with RT-qPCR. *indicates $p < 0.05$. CT, cycle threshold; OPS, oropharyngeal swab; SG, saline gargle.

patients (Figure 1A, column on the left). There were no significant differences in the CT values for the N gene or the Orf1/ab gene in SG and OPS samples (Figure 1A, column on the right). Nevertheless, the internal reference gene (RNase P) was significantly lower in SG samples than in OPS samples ($p < 0.0001$, Figure S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=128*). Likewise, the data on days 1-3 (Figure 1B), 4-7 (Figure 1C), and 8-14 (Figure 1D) exhibited the same trend, namely, there were no significant differences in the distribution or patients or CT values (SG *vs*. OPS). The subgroup analysis found no significant differences in the distribution of patients or CT values in symptomatic patients (Figure 2A). In asymptomatic patients, there were no significant differences in the distribution of patients (Figure 2B, column on the left). The CT values for the N gene ($p = 0.0463$) and Orf1/ab ($p = 0.0388$) in patients sampled with the SG were significantly lower than those in patients sampled with the OPS (Figure 2B, column on the right).

The current study verified the use of an SG to detect SARS-CoV-2. Both overall data and data on days 1-3, 4-7, and 8-14 revealed that the SG had satisfactory sensitivity in comparison to the most commonly used OPS (Figure 1). In symptomatic patients, there were no significant differences between the SG and OPS (Figure 2A). Interestingly, in asymptomatic patients, the CT values for the N gene and Orf1/ab were significantly lower in SG samples than in OPS samples, implying that the SG had better sensitivity (Figure 2B). To the extent known, this is the first study to compare the efficiency of an SG and OPS in patients infected with the Omicron variant, either symptomatic or asymptomatic. Findings regarding the efficiency of an SG agree with the results of previous studies using saline (*5,6*) and water (*7-10*). However, all of those studies detected older variants in an insufficient number of asymptomatic patients. The current study tested patients for the Omicron variant, and there was a large enough sample of asymptomatic patients. Results revealed that there were no differences between the SG and OPS in symptomatic patients. A point worth noting is that the results for asymptomatic patients seem to indicate that the SG had better sensitivity (lower CT values). A potential explanation might be that an SG collects more infected tissues/cells than a conventional OPS. In comparison to older variants, the Omicron variant mainly affects the upper respiratory tract. Due to the small sample in this pilot study, this aspect requires further investigation. Nevertheless, the results of this study provide inspiring evidence that an SG has satisfactory efficiency in comparison to an OPS in detecting the Omicron variant of SARS-CoV-2 that is currently prevalent. Moreover, SG sampling is easy to standardize. Indeed, it is a simple, convenient, less invasive, and easily adapted to self-sampling, so it is suited to mass, frequent, and repeated sampling to detect SARS-CoV-2.

Ethical statements

This study was strictly conducted per the guidelines of the Declaration of Helsinki of the World Medical Association (2000), and it was approved and supervised by the ethics committee of the Third People's Hospital of Shenzhen (approval number 2022-116-03). This study was registered with a Chinese clinical trial registry (ChiCTR2200063457). The study protocol was explained to all of the patients, who were asked to provide written informed consent to participation in this study.

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