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Short communication

Assessment of SARS-CoV-2 viral loads in combined nasal-and-throat swabs collected from COVID-19 individuals under the Universal Community Testing Programme in Hong Kong

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ABSTRACT

Background: Combined nasal-and-throat swabs (CNTS) is less invasive and easy to execute. CNTS also induces lower risk to healthcare workers upon collection. However, there is a lack of data on viral load assessment for population-wide testing.
Objective: This study assessed if CNTS is suitable as an alternative specimen type for the detection of SARS-CoV-2.
Methods: We assessed the viral load of SARS-CoV-2 in CNTS collected from COVID-19 individuals through the 2-week period of the Universal Community Testing Programme (UCTP) conducted in Hong Kong. In addition, we compared viral loads of SARS-CoV-2 for the paired CNTS and non-CNTS specimens among these individuals.
Results: This UCTP identified 48 COVID-19 individuals from nearly 2 million specimens collected. The viral loads of SARS-CoV-2 varied widely, cycle threshold values Ct 16.28–36.94, among symptoms and asymptomatic individuals. The viral loads for the paired CNTS and non-CNTS specimens were comparable.
Conclusions: This study demonstrated that CNTS could be a specimen of choice for diagnosis of SARS-CoV-2.

1. Introduction

The SARS-CoV-2 is a new type of coronavirus belonging to the genus *Betacoronavirus* and the species *Severe acute respiratory syndrome-related coronavirus* (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). Early detection of SARS-CoV-2 within a population is important for controlling and preventing the spread of COVID-19. In Hong Kong, two peaks of COVID-19 cases were observed before August 2020, namely during March–April 2020 and July–August 2020 (Mak et al., 2021a). In order to eliminate COVID-19 in the community, the Hong Kong Special Administrative Region (HKSAR) government launched the Universal Community Testing Programme (UCTP) on 1 September 2020. This UCTP was supported by the experts and technicians from Mainland China. The support included the preparatory works, arranging testing services suppliers and setting up facilities (The Government of the HKSAR, 2020a).

The combined nasal-and-throat swab (CNTS) was chosen as the specimen of choice for the UCTP. The samples were collected by trained medical or healthcare staff which were then delivered to a central testing facility set up specifically for the program (The Government of the HKSAR, 2020b). A total of 1.78 million specimens have been collected in UCTP between 1 September 2020 and 14 September 2020 (The Government of the HKSAR, 2020c). The Public Health Laboratory Services Branch (PHLSB) in Hong Kong has been designated as a WHO COVID-19 reference laboratory since April 2020 (WHO, 2021). It provides laboratory diagnostic services to public and private hospitals, and clinics in Hong Kong. All COVID-19 cases in Hong Kong were either diagnosed or confirmed by PHLSB. Before launching this UCTP, majority sample types received for SARS-CoV-2 confirmation by PHLSB were throat saliva, combined nasopharyngeal swab and throat swab (NPS and TS) and combined nasopharyngeal aspirate and throat swab (NPA and TS) (Mak et al., 2020). CNTS is a new type of specimen that was only received for confirmation through this UCTP.

The sampling method of UCTP is easy to execute and less invasive which might be useful for large scale population-wide testing. Although previous studies showed that CNTS might be used as an alternative sampling method to detect SARS-CoV-2, these studies were based on small groups of suspected individuals (LeBlanc et al., 2020; Vlek et al., 2021). The viral loads of COVID-19 individuals with the use of CNTS in a community-based setting were not known. This report characterized the viral loads of CNTS from the COVID-19 individuals identified in the UCTP. In addition, we compared the viral loads of CNTS and non-CNTS

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specimens obtained from these COVID-19 individuals to assess if CNTS is suitable as an alternative specimen type for the detection of SARS-CoV-2 for mass screening.

2. Materials and methods

2.1. Sample collection and preparation

CNTS that were preliminary tested positive for SARS-CoV-2 in the UCTP were sent to PHLSB for confirmation. Whenever individuals were tested positive for SARS-CoV-2, they were admitted to hospitals. Further respiratory specimens were collected for SARS-CoV-2 testing.

Laboratories were requested to send respiratory specimens to PHLSB for confirmation for different time intervals. RNA extraction was performed upon receipt of respiratory specimens. Respiratory specimens were kept at −70 °C freezers after RNA extraction.

2.2. SARS-CoV-2 detection

An in-house-developed RT-PCR assay was used to detect for the presence of SARS-CoV-2 nucleic acid in all samples. It targets the large polyprotein ORF1ab of SARS-CoV-2 (Mak et al., 2020). In brief, the RT-PCR was conducted using NxtScript Enzyme and Master Mix (Roche Diagnostics GmbH, Germany). Each test contained 5 μL RNA samples and 5 μL master mixture consisting of equal volume of forward primer, reverse primer and probe. The reverse transcription, amplification was performed in the LC480 System (Roche Diagnostics GmbH, Germany) with 40 amplification cycles. A designated reaction well was assigned for each specimen. The amplification curve for the test specimen should be in a sigmoidal shape with a cycle threshold (Ct) value not higher than 40. Viral RNA amount in respiratory specimens were estimated from Ct value. High Ct values mean that the respiratory specimens have low viral load and vice versa.

2.3. Statistical analysis

Descriptive statistics for continuous variables were presented as means. The *t*-test was used to compare the mean Ct values between groups. A *p*-value of <0.05 was considered as statistically significant.

3. Results

3.1. SARS-CoV-2 cases

Within the two-week period, between 1 Sep 2020 and 14 Sep 2020, the UCTP identified 48 COVID-19 individuals. The positive SARS-CoV-2 results of the CNTS specimens were tested twice. The results were reliable as detected by two different independent laboratories. Although negative results were not confirmed by us, the testing service providers had rich experiences in SARS-CoV-2 testing and rigorous quality assurance measures were implemented.

3.2. Viral load of CNTS specimens

For the 48 CNTS collected from 48 COVID-19 individuals, 17 (35.4 %) and 31 (64.6 %) were asymptomatic and symptomatic respectively. The difference in viral load in these two groups of individuals were statistically significant (unpaired t test, *p*-value = 0.023). The Ct values summary was shown in Table 1.

3.3. Viral load comparison between CNTS and non-CNTS specimens

A total of 19 non-CNTS specimens were collected from 18 COVID-19 individuals. The non-CNTS specimens were collected in hospitals within 2 days after the collection of the CNTS specimen. The non-CNTS specimens were NPS and TS (N = 10), throat saliva (N = 4), NPA and TS (N = 3), and nasopharyngeal swab (NPS) (N = 2). For these 19 paired specimens, the Ct values of 14 CNTS (73.7 %) were lower than non-CNTS samples. Comparisons of difference in Ct values for the four groups of pair-samples were not statistically significant, (1) CNTS vs NPS and TS, (2) CNTS vs throat saliva, (3) CNTS vs NPA and TS, (4) CNTS vs NPS. The pair numbers were arbitrary assigned from p01 to p19 according to the date of CNTS collection. The corresponding Ct values for these 19 paired specimens and difference in Ct values were shown in (Table 2).

Table 1
A summary of the viral load for combined nasal-and-throat swabs collected from the COVID-19 individuals in the Universal Community Testing Programme.

COVID-19 indiniduals	Number	Ct values	
		Range	Mean
Asymptomatic cases	17	24.26 - 35.96	30.43
Symptomatic cases	31	16.28 - 36.94	26.65
All cases	48	16.28 - 36.94	27.99

4. Discussion

The UCTP achieved the target of early identification of COVID-19 individuals in the community and provided the opportunity to abort potential community transmission chains. Our routine surveillance system might miss COVID-19 cases due to mild symptoms or asymptomatic individuals who would most unlikely present to the healthcare system (Lam et al., 2020). Although the viral load of asymptomatic cases were lower than symptomatic cases in general, the highest viral load among the asymptomatic cases was Ct 24.26 as shown in the present study. It is likely that silent transmission existed in this case and might have played a role in the local transmission chain. In addition, there is insufficient evidence that COVID-19 cases with high Ct values are not infectious (CDC, 2021). Successful COVID-19 control could only be

Table 2
The viral load comparison between combined nasal-and-throat swabs and non-combined nasal-and-throat swabs collected from the COVID-19 individuals in the Universal Community Testing Programme.

Pair	Ct values[a]		Ct difference
CNTS and NPS and TS group	CNTS	NPS and TS	
- p02	31.91	37.00	5.09
- p03	22.29	23.74	1.45
- p04	36.28	37.95	1.67
- p07	24.75	25.44	0.69
- p08[b]	33.86	32.61	−1.25
- p11	20.74	22.55	1.81
- p12	26.78	31.59	4.81
- p14	28.94	33.71	4.77
- p17	31.68	31.84	0.16
- p19	24.34	20.66	−3.68
CNTS and throat saliva group	CNTS	throat saliva	
- p09[b]	33.86	37.37	3.51
- p10	28.65	34.56	5.91
- p15	18.39	16.70	−1.69
- p16	17.89	20.77	2.88
CNTS and NPA and TS group	CNTS	NPA and TS	
- p05	33.68	35.74	2.06
- p13	20.21	23.72	3.51
- p18	32.23	21.35	−10.88
CNTS and NPS group	CNTS	NPS	
- p01	35.96	32.77	−3.19
- p06	29.62	33.85	4.23

[a] CNTS, combined nasal-and-throat swabs; NPS and TS, combined nasopharyngeal swab and throat swab; NPA and TS, combined nasopharyngeal aspirate and throat swab; NPS, nasopharyngeal swab.
[b] p08 and p09 were obtained from the same patient.

achieved by widespread testing, contacts tracing and cases isolation.

The preferred specimens for molecular testing of respiratory viruses are collected from upper respiratory tract such as NPA or NPS. The molecular testing of COVID-19 is no exception (McIntosh et al., 1993; Marty et al., 2020). There are situations where collecting of such specimens can be problematic, such as mass screening. Collection methods that are less invasive and induced lower risk of exposure to others upon collection were highly desirable (WHO, 2020). CNTS can act as alternative specimen type which is less invasive and easier to perform and at the same time yielded similar sensitivity for the detection of major respiratory viruses (Lambert et al., 2008). One study showed that CNTS was even better than NPA for diagnosing H1N1pdm09 (de la Tabla et al., 2010). In the present study, our data showed that both asymptomatic and symptomatic COVID-19 patients can be identified using CNTS with wide range of viral loads (Ct 16.28–36.94). In addition, the viral load of CNTS were comparable to the paired non-CNTS specimens. Our results were concordant to previous studies that CNTS yielded a similar sensitivity in the detection of SARS-CoV-2 when comparing with other respiratory specimens (LeBlanc et al., 2020; Vlek et al., 2021). A recent review summarized 46 studies worldwide demonstrated that CNTS shared similar performance with other respiratory specimens for diagnosis of SARS-CoV-2 (Lee et al., 2021). These results showed that CNTS is a suitable alternative specimen type for the detection of SARS-CoV-2.

RT-PCR is the gold standard and the most widely used method to detect SARS-CoV-2 (Carter et al., 2020; Vandenberg et al., 2021). Its diagnostic efficacy, however, depends on multiple factors. Besides specimen types, collection media (Kline et al., 2021), extraction systems (Ambrosi et al., 2021) and PCR assays (Vogels et al., 2020) can also directly affect the overall results. Currently, RT-PCR, antigen and antibody tests are the most widely used techniques. Other advances techniques such as droplet digital PCR should be explored in future to enhance the detection rate of SARS-CoV-2 (Falzone et al., 2020; Suo et al., 2020; Falzone et al., 2021). Performance comparison between those different techniques is beyond the scope of this study.

The limitation of this study includes a relatively short time period in a community setting. Other limitation is the small number of COVID-19 individuals as well as positive samples. The low prevalence was probably related to the stringent measures implemented in the second peak of COVID-19 cases during July–August 2020. Finally, due to the role of PHLSB in this study, we confirmed preliminary SARS-CoV-2 positive CNTS specimens, sensitivity and specificity of using CNTS for diagnosis of SARS-CoV-2 could not be obtained. However, we performed a small study to show the usefulness of CNTS for diagnosis of SARS-CoV-2 with the use of RT-PCR and antigen techniques (Mak et al., 2021b).

In summary, our data showed that CNTS is not inferior to other respiratory specimens and have direct clinical application which will be useful for pandemic planning. CNTS could be a specimen of choice in our setting to perform SARS-CoV-2 and other respiratory viruses surveillance.

Data availability

Data sharing is not applicable to the current study as the data are comprehensively described throughout this article.

CRediT authorship contribution statement

Gannon C.K. Mak: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Anita Y.Y. Ng:** Validation, Investigation, Supervision. **Edman T.K. Lam:** Supervision. **Rickjason C.W. Chan:** Supervision, Writing - original draft, Writing - review & editing. **Dominic N.C. Tsang:** Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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