Effect of Sampling Force on The Quality of Oropharyngeal Swabs

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Abstract

Background

The aim of this study was to clarify the most suitable sampling force for OP swabs.

Methods

Thirty healthy subjects were continuously included in this study. The quantitative relationship between sampling force and the quality of OP swabs (C_T values of GAPDH in OP swab specimens) has been explored.

Results

No significant relationships between the median sampling forces and qualities of OP swab were found in this study ($r=-0.079$, $P=0.547$). The median and maximum sampling forces were remarkably differed from different sampling doctors ($P<0.001$). However, the mRNA expression of GAPDH of OP swabs specimens that were taken by two different doctors showed no statistical difference. The mRNA expressions of GAPDH presented no significant difference among three groups (low level: 0–20 g, middle level: 20–40 g, high level: > 40 g) of sampling force ($P=0.873$). However, it was observed that the incidence of side effects was significantly increased in the middle and high level groups, compared to the low level group ($P<0.002$).

Conclusions

We believed that a sampling force ranged from 0 to 40 g was considered as the optimal strength during OP swab sampling.

Introduction

Oropharyngeal (OP) swabs has been described as a frequently-used important methodology for the rapid differentiation and accurate identification of potential respiratory pathogens in adults, which ultimately leads to the effective early diagnosis and therapy. Standard sampling of OP swab, including the precise delivery to target tissue, appropriate force and touch avoidance of surrounding tissue, has been confirmed to be prerequisite for the enhancement of positive detection rate^[1–3]. However, there is no recognized criterion to evaluate the sampling force. In fact, the understanding of sampling force differs between individuals. And the inappropriate sampling force may lead to damage of surrounding tissues and/or inconsistent quality of OP swabs.

The aim of this study was to clarify the most suitable sampling force for OP swabs by demonstrating the quantitative relationship between sampling force and the quality of OP swabs. In addition, the adverse effect of the inappropriate use of sampling force was also discussed in the current study.

Methods

Subjects

Thirty healthy subjects were continuously included in this study from March 6 to March 8, 2020 in the First Affiliated Hospital of Guangzhou Medical University.

Acquisition Of Op Swabs

A pressure sensor (Futek LSB200, Futek Advanced Sensor Technology, U.S.A.), which recorded the pressure data with a specific application in the computer, was employed to the acquisition of OP swab (shown in Fig. 1). Paired OP swab specimens of each participant were independently obtained by two experienced medical doctors (group A and group B). The interval sampling time was 20 minutes. A rayon swab was inserted into oropharynx and stroked twice on the posterior wall of the oropharynx under direct visualization. After sampling, the swabs were then put in a separate sterile test tube containing 1 ml of virus preservation solution.

The indicator for the safety evaluation of the sampling was the incidence of adverse reactions. After OP swab sampling, each participant was evaluated to determine their overall sensation during sampling and to detect the presence of congestion and damage of the throat, nausea, vomiting and pain.

Detection Of A Housekeeping Gene

The quality of swabs was determined by the cycle threshold (Ct) value for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as described previously ^[4], where a lower Ct value suggested more collected cells, and better swab quality. An oropharyngeal swab with a Ct value of ≤ 37 was considered as a qualified specimen, and an oropharyngeal swab with a Ct value of > 37 was considered as a failed specimen.

Statistical analysis

The statistical software SPSS 22.0 was used for statistical analysis. Continuous normally distributed variables were presented as means $\pm$ standard deviations and analyzed using independent sample t-tests. A Wilcoxon test was used to compare paired data. Continuous non-normally distributed variables were described as medians and interquartile ranges (IQRs) and assessed using a Mann-Whitney U test. The categorical variables were expressed as frequencies and percentages and evaluated using a Chi-squared

test or Fisher's exact test. The associations between the different quantitative variables were estimated using Pearson's correlation coefficient. A two-sided P value less than 0.05 was considered statistically significant.

Results

General demographic feature of healthy subjects

A total of 30 healthy subjects in the First Affiliated Hospital of Guangzhou Medical University, 12 males and 18 females, aged from 23 to 65 years, were enrolled in this study. This study found no evidence of a gender bias regarding the C_T values of GAPDH in OP swab specimens (27.3 ± 1.5 in males vs 27.0 ± 2.2 in females, $P = 0.669$). In addition, there were no significant associations between ages and the C_T values of GAPDH ($P = 0.408$).

Associations between sampling forces and the qualities of OP swabs

The sampling force, which was the non-normally distributed variable (Kolmogorov-Smirnov test, $P < 0.05$), was presented as median and IQRs. The maximum of sampling force was also documented in the current study. In addition, the positive association between the median and maximum sampling force was identified ($r = 0.72$, $P < 0.001$) (shown in Fig. 2). However, no significant relationships between the median sampling forces and qualities of OP swab (the C_T values of GAPDH) were found in this study ($r = -0.079$, $P = 0.547$) (shown in Fig. 3).

Effect of different sampling medical doctors on the quality of OP swabs

The median and maximum sampling forces were remarkably differed from different sampling doctors ($P < 0.001$) (Table 1). However, the mRNA expression of GAPDH of OP swabs specimens that were taken by two different doctors showed no statistical difference, indicating the qualities of OP swabs were irrelevant to the sampling forces. Regarding the side effects of unsuitable sampling force, group B showed more obvious adverse effects than group A (7/30 vs 0/30, $P = 0.011$) (Table 1). In fact, 7 out of the 30 participants (23.3%) in group B had side effects, with nausea (71.0%) being the most common, followed by pain (28.6%) (Table 2).

Table 1
The quality and adverse effect of OP swab specimen obtained by two different doctors

	Group A	Group B	<i>P</i>
	N = 30	N = 30	
Median force (g)	52.7 ± 24.4	132.6 ± 35.7	<0.001
Maximum force (g)	11.4 ± 4.7	37.7 ± 11.2	<0.001
CT values of GAPDH	27.3 ± 1.9	27.0 ± 2.0	0.578
Adverse effect	0%(0/30)	23.3(7/30)	0.011

Table 2
Adverse effect in two groups

Adverse effect	Group A	Group B
Nausea	0	71%(5/7)
Pain	0	28.6%(2/7)
Others	0	57%(4/7)
Congestion of throat	0	0(0/7)
Damage of throat	0	0(0/7)

Effect of different ranges of sampling force on the result of OP swabs

Three groups(low level: 0–20 g, middle level: 20–40 g, high level: > 40 g)of sampling force were classified in this study. The mRNA expressions of GAPDH presented no significant difference among three groups ($P=0.873$) (shown in Fig. 4). However, it was observed that the incidence of side effects was significantly increased in the middle and high level groups, compared to the low level group ($P<0.002$) (Table 3).

Table 3
Comparison of adverse effect in different sampling force groups

Groups	The proportion of adverse effect(%)
Low level group (0-20g)	0 (0/30)
Middle level group (20-40g)	15 (3/20)
High level group (>40g)	40 (4/10)

Discussion

Effect of sampling force on the quality and adverse reaction of OP swabs was explored for the first time in this study. It has been confirmed that sampling force during the acquisition of OP swabs was irrelevant to the quality of OP swabs. A sampling force less than 40 g was considered as most appropriate force because of the mild and infrequent adverse effect. Based on this theory, a new device was developed for measuring force during OP sampling (Fig. 5, Patent application number: 2020102086110). Training for healthcare workers with this new device can be used to further standardize the OP swabs sampling.

Since December 2019, the global spread of highly pathogenic SARS-COV-2 has become a worldwide concerned issue^[5]. OP swabs has been recommended upper respiratory tract specimen types for the detection of SARS-COV-2 and other respiratory virus and mycoplasma^[6–8]. In addition, a standardized approach to OP swab handling, collection, processing, storage and analysis has been reported to be essential for rapid differentiation and accurate identification of potential respiratory pathogen^[2,9–10], which eventually lead to timely and appropriate measures for public safety. Chemical and physical characteristics differ from various tip materials of OP swabs, which might result in the inconsistent quality of OP swabs^[11]. Flocked nylon swab has been shown to be superior to the cotton swab for the increases contact area, thereby providing strong experimental support (acquisition of more epithelial cells) for pathogen exploitation^[12]. Flocked nylon swab has also been shown to transfer 20–60% more micro-organisms from their surfaces than other swabs^[12]. However, the combination of cotton swab sampling and QIAcube system showed an advantage in the identification of certain viral pathogens over other swabs⁹. Besides, there was influence of different sampling sites on the detection of potential pathogens^[13–14].

However, the contribution of sampling force to the evaluation of respiratory pathogens has not yet been reported. Inappropriate sampling force of OP swabs sometimes happens due to the understanding of sampling force differs between individuals. In the current study, the quality of OP swabs was irrelevant to the different sampling forces and collectors. The incidence of adverse effect, including gag reflex, pain and other discomfort, increased linearly as the sampling force increased. In addition, 40% of the recruited subjects whose sampling force was more than 40 g experienced side effects. However, it is difficult to control the sampling force without a device which can precisely verify and measure the strength of sampling force. Therefore, a new device was developed for measuring force during OP sampling. The device would show green color with a sampling force ranged from 0 to 40 g and present red color with a sampling force more than 40 g. (shown in Fig. 5).

There were limitations in this study. The sample size was small. Only three interval values of sampling force and one housekeeping gene were detected in this study. A large sample size study with more interval values of sampling force should be performed to confirm the results of this study.

Conclusions

In conclusion, we believed that a sampling force ranged from 0 to 40 g was considered as the optimal strength during OP swab sampling. The new device, which measure sampling force of OP swab in the

current study, can be helpful to further enhance the efficiency and robustness of OP swabs.

Abbreviations

OP

oropharyngeal; Ct:cycle threshold; GAPDH:glyceraldehyde 3-phosphate dehydrogenase;

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (approval number: 2020-046). Written informed consent was signed by each participant at the time of enrollment. Data were analyzed and interpreted by the authors.

Consent for publication

Not applicable.

Competing interest

The authors have declared no conflicts of interest and alone are responsible for the content and the writing of the manuscript.

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Not applicable.

Author Contributions

All the authors contributed to study design, data collection, and manuscript writing/review.

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Availability of data and materials

Not applicable.

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Figures

Figure 1

A general introduction to the measurement of sampling force, blue arrow: pressure sensor.

Figure 2

Analysis of the association between the median and maximum sampling force.

Figure 3

Analysis of the association between the median sampling force and CT value of GAPDH.

Figure 4

Comparison of CT value of GAPDH in different sampling force groups.

Figure 5

A new device for the detection of sampling force, blue arrow: indicator lights, green color: 0-40 g, red color: > 40 g.