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The sampling efficiencies of commercial nasopharyngeal swabs

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ABSTRACT

Upper respiratory tract samples are the most commonly used samples for coronavirus disease 2019 (COVID-19) diagnosis. The samples collected from the nasopharynx are preferred for viral nucleic acids detection. Commercial nasopharyngeal swabs (NPSs) are the major factor that influences the sampling quality. We here evaluated the acceptability and efficiency of NPSs from five manufacturers by examining the concentration of glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) retrieved from the swabs using the RT-PCR method. Significant different concentrations of GAPDH were detected, ranged from 4.36×10^8 copies/mL to 6.98×10^{10} copies/mL among the five swabs ($P < 0.05$). The designation of the swab head, with or without tip expansion, had limited influence on the collection efficiency. The discrepancy among the NPSs emphasized the improvement of the swab head material.

1. Introduction

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has ravaged the world, compelling the World Health Organization (WHO) to declare it as a global pandemic on 11 March 2020, caused more than 263 million confirmed cases and 5.23 million deaths till 3 December 2021 [1]. The laboratory-based viral nucleic acid testing is the principal evidence for early confirmation of SARS-CoV-2-infected cases. The sampling procedures are the main factors influencing the sensitivities of nucleic acid testing, including anatomic sampling site, transportation, transient storage conditions, and *etc*. Respiratory tract samples for viral diagnostic tests could be taken from the upper respiratory tract, such as NPSs, oropharyngeal swabs and saliva, or lower respiratory tract, including those of sputum and bronchoalveolar lavage. The lower respiratory tract specimens are commonly collected in symptomatic or severe COVID-19 cases [2–4]. For patients with mild symptoms or population nucleic acids screening, upper respiratory samples are preferred to be collected. NPS is recommended for SARS-CoV-2 screening to acquire more reliable results than saliva, nasal, and oropharyngeal swabs [2,5–8]. The currently available commercial consumables of NPSs are significant factors influencing the sampling quality. The designation of the swab is different in the shape, diameter, length of the swab head. Flocking material is commonly used on swab heads. As the anatomic site of nasopharynx is very sensitive, the incorrect sampling process may cause tissue damage, bleeding, or irritation. The comfortability of NPSs sampling would also be considered.

This study evaluated the sampling efficiency of five widely used commercial NPSs; one was COPAN flocked swab, the other four swabs were manufactured in China. The designation, individual comfortability, and cost-effectiveness are also compared.

2. Materials and methods

2.1. NPSs characteristics

Five brands of NPSs, with the code number 1, 2, 3, 4, 5, were selected. Code 1 was COPAN flocked swab (Cat:503CS01, Brescia, Italy). Code 2 to 5 were manufactured in China, with catalog numbers MSF-96000BQ, BZ0302-1, ST001-2, CY-96000 in order. All the heads of the swabs were flocked. The characteristics of NPSs were recorded, including the shape, diameter, and length of the swab head, breakpoint from the head, and expansion of the swab head.

2.2. Sampling process and evaluating the comfortability

A total of 33 volunteers were randomly recruited, and they had no known respiratory tract symptoms or nasal and pharyngeal illness. The median age of the participants was 26 (21–48) years old (interquartile

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range [IQR]); 18 (55%) were female. The sampling procedure was performed by the same person trained well on samples collection. The swab was inserted randomly into either right or left naris, then gently rotated for several seconds at the site of the nasopharynx, then kept in the viral transport medium (VTM). Five kinds of NPSs for each volunteer were tested, alternating the two nostrils at one-hour intervals. The comfortability of each swab was evaluated as a score, which included pain (usually-, slightly+, very painful++), soreness (usually-, slightly+, very painful++), and causing tear (no-, less+, tears++). Volunteers selected the most comfortable and uncomfortable swabs then summarized the frequency for each swab.

2.3. Efficiency of NPSs

The efficiency of sampling with different NPSs was evaluated by testing the concentration of the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) retrieved from the swabs by using the PCR method. A total of 400 μL VTM extracted nucleic acid using the chemagic™ 360 instrument (PerkinElmer, USA). The primers and probe for detecting GAPDH were used as follows: the forward primer (5′-CCAGGTGGTCTCCTCTGAC-3′), the reverse primer (5′-CACCCTGT TGCTGTAGCCA-3′), and the probe (5′-HEX-CATTGCCCTCAACGAC CACT-BHQ1-3′) [9]. The reaction mix was 20 μL, including five μl of 4× Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania), 0.1 μL of 50 μM probe, 0.2 μL each of 50 μM forward and reverse primers, 12.5 μL of nuclease-free water, and two L of extracted nucleic acid. PCR reactions were carried out by using Bio-Rad instrument (Bio-Rad CFX96, Hercules, CA, USA), with the conditions of 15 min at 50 ℃ for reverse transcription, 10 min at 95 ℃ for pre-denaturation, followed by 45 cycles of 15 s at 95 ℃ and 1 min at 60 ℃. The copy numbers of GAPDH were measured and quantified using the GAPDH RNA standard curve to decide the efficiency of nucleic acid recovery. Comparisons between groups were made by using t-tests. Two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were conducted by using GraphPad Prism software version 9.0.

3. Results

3.1. Comparison of the appearance of the commercial NPSs

The commercial NPSs consisted of flocked swab heads and plastic rods with a breakpoint. A significant red line was marked on the breakpoint of Code 1 plastic rod, and the others had no marker on the breakpoint (Fig. 1). The parameters, as well as the price for each swab, were summarized in Table 1. The NPSs showed different appearances on total length, length of the breakpoint from the head, head length, head diameter, and expansion of the swab head. Code 1 was the easiest to break at the breakpoint, followed by Code 5 and Code 3, Code 2, and Code 4. The softness of the plastic rod was tested, and Code 1 swab was the best, followed by Code 5, Code 2, Code 4, and Code 3.

3.2. Comfortability of the NPSs

The volunteers decided the testing order of the swabs in a single-blinded way. After all the swabs were finished to be tested, the volunteer decided the comfortability. All the volunteers (39.39%) complained code 2 was the most uncomfortable one, while code 5 was the most comfortable. The comfortability of the NPSs varied considerably (Table 2). The shape and tip expansion of the NPSs head had limited impact on the comfortability of different codes (Table 1).

3.3. Sampling efficiency of NPSs

Nucleic acid was extracted from the NPSs, and the concentrations of GAPDH (copies/mL) were tested using RT-PCR to evaluate the sampling efficiency of the NPSs. As shown in Fig. 2, the concentration of GAPDH for Code 1 and Code 2 swabs were significantly higher than Code 5 ($P = 0.025$ and $P = 0.036$), indicating retrieving efficiency of Code 1 and Code 2 swabs. The fluctuation range of the GAPDH concentrations displayed a stable collection efficiency of the Code 1 swab. For the Code 2 swab, a significant GAPDH concentration fluctuation was observed, indicating an unstable collection efficiency. Our findings suggested that the head material of the swabs should be the most influential factor in sampling efficiency.

4. Discussion

During the control and prevention of COVID-19, swabs are the most commonly used materials to collect samples and screen the pathogens. Current knowledge considers that the NPS's sensitivity is higher than that of the oropharyngeal swab in the early identification of the COVID-19 case [2,5,6,10]. The NPS contains flocked swabs and traditional fiber swabs, and the flocked swabs are better than conventional fiber swabs on sampling efficiency [11,12]. In this study, we compared the comfortability, and sampling efficiency of the commercial flocked NPSs. The sampling procedure was performed by one person who had been trained well in case of the sampling bias to eliminate human factors during sampling. The volunteers without symptoms of respiratory tract infections were randomly recruited. Each volunteer was informed only swabs code number, and they decided the sequence of the swabs and the inserted naris when sampling. The results showed that four of the NPSs had similar retrieving efficiency according to the concentration of GAPDH. In contrast, one domestic manufacturer showed lower and unstable sampling efficiency than others.

During the outbreak of COVID-19, swabs were the most commonly used consumables. Three dimensions printed NPSs have been commercially available to support the prevention and control of COVID-19 [13,14]. The preclinical evaluation of NPSs includes swab design, collection sufficiency, and PCR compatibility[14]. Our findings showed that the tip expansion of the swab head had limited impact on the collection efficiency based on the concentration of GAPDH retrieved from the swabs. The optimization of swab head material should be considered.

NPS sampling is a critical step for viral nucleic acids detection. Non-qualified sampling procedure may make diagnostic errors, especially when patients have very low viral loads [15]. When performing nasopharyngeal sampling, rotation of the swab upon the nasopharynx has no significant improvement for sampling quality; rotation is also less tolerable for participants [16]. If the insertion depth is not enough, mid-nasal or mid-turbinate samples but not NPS would be collected, potentially reducing the sensitivity of detections [17].

The sampling procedure using NPS was easy to be handled, and the comfortability was acceptable. However, the inconsistent sampling efficiency of NPSs of the five manufacturers indicated an urgency to standardize the evaluation and manufacture procedure. The discrepancy among the NPSs emphasized the improvement of the swab head material. The main limitation of our study was the small sample size and limited kinds of swabs. Therefore, further evaluation on the quality of the consumable of NPSs should be performed based on a larger scale sample size.

Ethics statement

The Ethical Review Committee approved this study at the Institute of Pathogen Biology, Chinese Academy of Medical Sciences. The ethics

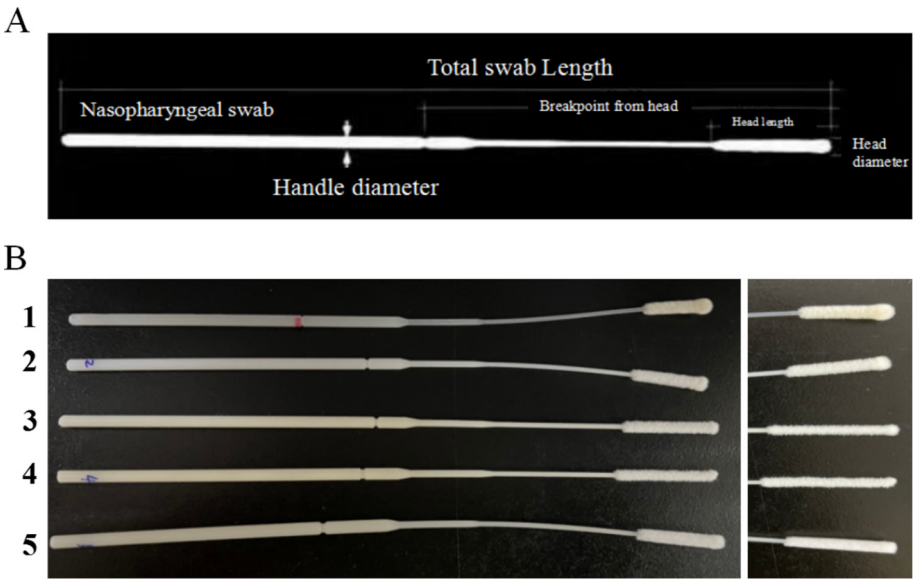

Fig. 1. The appearance and the head of nasopharyngeal swabs. A) Parameters of nasopharyngeal swabs. B) The appearance of the tested swabs codes 1 to 5 (From top to bottom).

Table 1
The parameters of nasopharyngeal swabs.

Code	Total length (mm)	Handle diameter (mm)	Length of the breakpoint from head (mm)	Head length (mm)	Head diameter (mm)	Expansion of swab head	Price (RMB, yuan)
1	150	2.5	100	14	2.5	Yes	6.3
2	148	2.5	80	17	3	Yes	0.5
3	150	2.5	80	22	2	Yes	1.2
4	150	2.5	75	22	3	No	0.3
5	148	2.5	90	22	3	Yes	1.2

Table 2
The number of volunteers decided the comfortability of each nasopharyngeal swab.

Comfortability	Code 1	Code 2	Code 3	Code 4	Code 5
High	4 (12.12%)	4 (12.12%)	8 (24.24%)	4 (12.12%)	13 (39.39%)
Low	6 (18.18%)	13 (39.39%)	5 (15.15%)	5 (15.15%)	4 (12.12%)

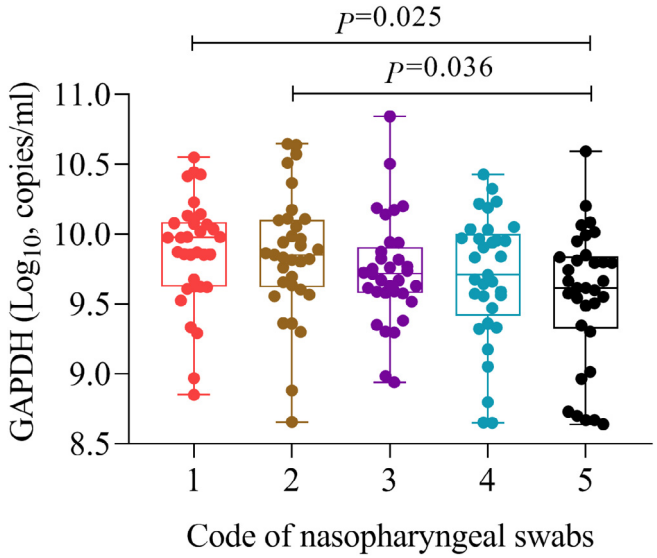

Fig. 2. The nucleic acid collection efficiency of nasopharyngeal swabs.

statement number is IPB-2020-16. Each participant provides signed written informed consent.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Ying Wang: Samples Collection, Data Analysis, Writing – Original Draft. **Geng Wang**: Samples Collection. **Lulu Zhang**: Formal Analysis. **Lili Ren**: Conceptualization, Data Curation, Formal Analysis, Writing –

Review & Editing, Funding Acquisition. **Jianwei Wang**: Conceptualization, Writing – Review & Editing.

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