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Performing nasopharyngeal swabs—Guidelines based on an anatomical study

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Abstract

Nasopharyngeal swabs are performed to collect material for diagnosing diseases affecting the respiratory system, such as Covid-19. Yet, no systematic anatomical study defines concrete prerequisites for successfully targeting the nasopharyngeal mucosa. We therefore aim at simulating nasopharyngeal swabs in human body donors to characterize parameters allowing and supporting to enter the nasopharynx with a swab, while avoiding endangering the cribriform plate. With the aid of metal probes and commercial swabs a total of 314 nasopharyngeal swabs in anatomical head/neck specimens stemming from 157 body donors were simulated. Important anatomical parameters were photo-documented and measured. We provide information on angles and distances between prominent anatomical landmarks and particularly important positions the probe occupies during its advancement through the nares to the upper and lower parts of the nasopharynx and cribriform plate. Based on these data we suggest a simple and safe three-step procedure for conducting nasopharyngeal swabs. In addition, we define easily recognizable signals for its correct performance. Evaluations prove that this procedure in all specimens without deformations of the nasal cavity allows the swab to enter the nasopharynx, whereas a widespread used alternative only succeeds in less than 50%. Our data will be the key for the successful collection of nasopharyngeal material for detecting and characterizing pathogens, such as SARS-CoV-2, which have a high affinity to pharyngeal mucosa. They demonstrate that the danger for damaging the cribriform plate or olfactory mucosa with swabs is unlikely, but potentially higher when performing nasal swabs.

KEYWORDS
anatomy, Covid-19, nasopharynx, respiratory, SARS-CoV-2

1 | INTRODUCTION

Material from the mucosa of the nasopharynx is collected by means of nasopharyngeal swabs. The procedure is very simple and chiefly used for diagnosing and characterizing suspect respiratory infections (Babady et al., 2018; Gritzfeld et al., 2011; Kaufman et al., 2020; Lieberman et al., 2006; Wang et al., 2020). Recently the method has attracted enormous attention, since it is used to collect material for testing for and characterizing the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes Covid-19 (CDC, 2020; Mawaddah et al., 2020; Piras et al., 2020; Seo et al., 2020; WHO, 2020; Zou et al., 2020).

A long shaft, made of wood, plastic, or similar materials with a tip covered in polyester, rayon, cotton wool, or flocked nylon is inserted into one of the nares. Its tip is then pushed posteriorly through the nasal cavity and the choana to the backside of the pharynx. Here mucus and mucosal cells are collected by swabbing with the material covering the tip.

For educating medics and paramedical staff to perform nasopharyngeal swabs, a large number of videos, pictograms, schematic representations, and verbal descriptions were made available in modern digital media (CDC, 2020; Falcone, 2020; Kaufman et al., 2020; Marty et al., 2020; NMC, 2020; Pondaven-Letourmy et al., 2020; Sananès et al., 2020). They are based on traditional representations of the upper respiratory tract as displayed in anatomical and clinical textbooks (Drake et al., 2015; Logan et al., 2016; Netter, 2019; Sobotta et al., 2008). For estimating the position and topology of the palate, choana, and nasopharynx from external and thus for guiding the direction in which a swab has to be inserted and advanced, simple anatomical landmarks were suggested. The most important is the ear and the opening of the external acoustic meatus (CDC, 2020; Falcone, 2020; Marty et al., 2020; NMC, 2020) and it is advised to insert swabs toward these structures for a depth of 7–10 cm (Falcone, 2020; NMC, 2020; Pondaven-Letourmy et al., 2020).

The widespread availability of guides describing the swab procedure is in strong contrast to the lack of a sound anatomical justification of potential insertion directions. We therefore set out to carefully characterize and evaluate important landmarks and parameters required for guiding correct and successful advancement of a swab to the nasopharynx. In addition, we aimed at objectively defining the precise direction, in which a swab should be arranged to directly target the nasopharyngeal mucosa without endangering the cribriform plate of the ethmoid bone.

2 | MATERIALS AND METHODS

Both sides of 157 (85 females, 72 males) head/neck specimens, severed from the bodies of human body donors at the atlanto-occipital transition, were examined. Mean age at time of death was 82 years (50–108). All specimens were derived from Caucasians. They were perfused with 1% formaldehyde/4% carbol for an average of 12–16 h, immersed in 2% formaldehyde/2% carbol for at least 8 months and then pre-dissected during students' dissection classes. In 110 head/neck specimens nasopharyngeal swabs were simulated with a metal probe with a tip of 2 mm and the success was evaluated by observing the position of its tip from dorsocaudal after opening the posterior walls of the pharynx. Forty-seven specimens were sawed in half in the mediansagittal plane after the soft tissues were cut with scalpel and scissors and nasopharyngeal swabs were simulated at the 94 halves.

To indicate anatomical landmarks, pins were inserted at the nasion, subnasale, and mid of tragus. Then the probe was placed and anatomical parameters were measured at following probe positions: (a) Placed upwards along the soft tissue cushion at the posterior nares; (b) Placed along the hard palate with the tip touching the posterior wall of the pharynx; (c) Placed to touch both, the soft tissue cushion at the posterior nares and the fornix pharyngis; (d) Placed to touch the soft tissue cushion at the posterior nares and the cribriform plate (Figure 1). The latter was performed in mediansagittal sectioned specimens only. In each position distances were measured with scales and digital images were captured from strictly lateral and either caudal or medial by using a camera (Canon EOS 550D) on a stative. Using the Paint (Microsoft Corporation, Version 6.1) and ImageJ (Fiji 2011) software packages, lines were drawn in the digital images, which connected the pins at the subnasale and nasion and the subnasale and tragus respectively. Then the angles between the probe and both lines were measured (Figure 1). Statistics were performed using MS Excel 2016 (Microsoft Corporation) and SPSS (IBM, Version 26.0). Student's *t*-test was used for assessment of differences in measurement between the left and right sides of specimens (paired) as well as sex-specific variability (independent). All tests were two-tailed at $\alpha = 0.05$, and the Bonferroni correction was applied in case of multiple testing.

The study was in accordance with the declaration of Helsinki and local ethic regulations. All specimens were obtained from body donors, who volunteered and gave written consent to donate their dead bodies to the Division of Anatomy of the Medical University of Vienna for teaching and science prior to decease.

3 | RESULTS

In 152 of the 157 head/neck specimens the tip of the probe was successfully and smoothly advanced through the nares and the choana and touched the posterior wall of the pharynx. In 2 (1.27%, 1 female and 1 male) the advancement of the probe/swab was blocked bilaterally, and in 3 (1.91%, 1 female and 2 males) unilaterally (once on the right and twice on the left). These specimens were excluded from statistics.

The mean angle between the probe inserted along the palate and the subnasale/nasion line (αA) measured 82.9° (69–96.5); the mean angle with the subnasale/tragus line (βA) 9.3° ((−2)–17.6); the average distance between the posterior lower rim of the nares and the backside of the pharynx 8.7 cm (7.3–10.5) (Figures 2(A),(D) and 3(A)).

The mean angle between the probe inserted to touch the fornix pharyngis and the subnasale/nasion line (αB) measured 76.3° (63–90.5); the mean angle with the subnasale/tragus line (βB) 0.8° ((−10)–14) (Figure 2(B),(E)).

The mean angle between the probe inserted to touch the cribriform plate and the subnasale/nasion line (αC) measured 36.7° (29.5–48); the mean angle with the subnasale/tragus line (βC) -35.4° ((−45.5)–(−25.5°)); the average distance between the posterior lower rim of the nares and the cribriform plate 6.1 cm (5.0–7.7) (Figures 2 (C),(F) and 3(B)).

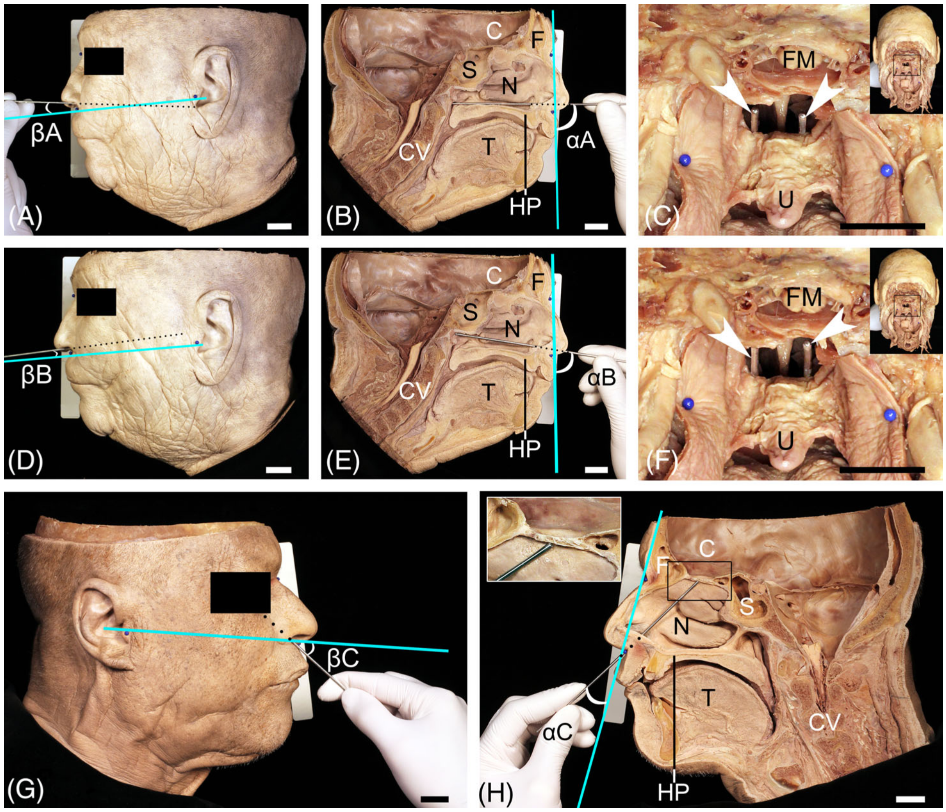

FIGURE 1 Probe positions in respect to anatomical landmarks. (A)–(C). Positioned along the hard palate (HP). (D)–(F). Positioned to pass immediately below the upper rim of choana. (G, H) Positioned to touch the cribriform plate. View from lateral (A, D, G), medial (mediansagittally sectioned head/neck specimens) (B, E, H), and dorsocaudal (full head/neck specimens) (C, F). Arrowheads point to probe; blue pins pin the posteriorly opened pharynx wall laterally. C, anterior cranial fossa; N, nasal cavity; HP, hard palate; F, frontal bone; S, sphenoid bone; T, tongue; CV, bodies of cervical vertebrae; FM, foramen magnum; U, uvula; α, angle between probe and line connecting subnasale and nasion; β, angle between probe and line connecting subnasale and tragus. Scale bars, 2 cm

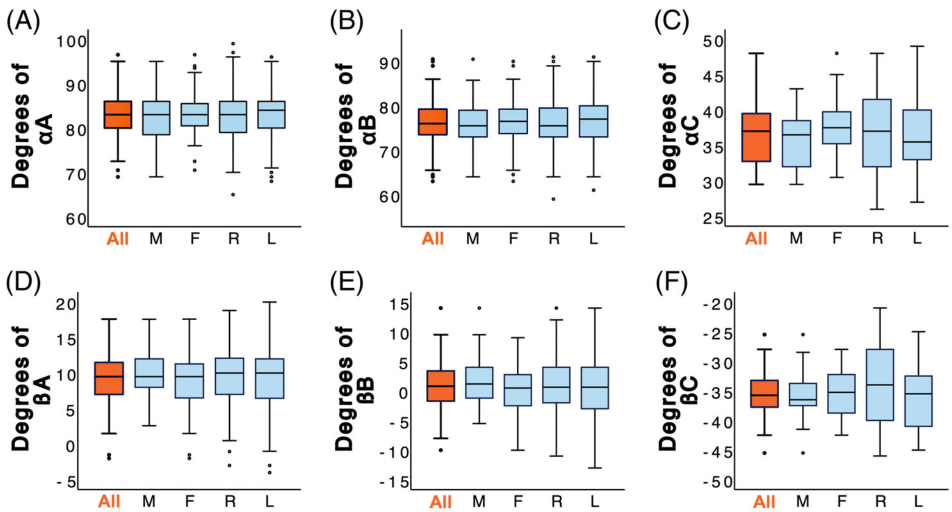

FIGURE 2 Angles. (A)–(C) Angles α between probe and line connecting subnasale and nasion (compare Figure 1(B),(E),(H)). (D)–(F) Angles β between probe and line connecting subnasale and tragus (compare Figure 1(A),(D),(G))

Statistical analysis revealed no differences in angle measurement between left and right and between females and males. Also, all measured distances were statistically equal between left and right. Yet, the distance between the posterior lower rim of the nares and the backside of the pharynx ($p < 0.001$) and the distance between the posterior lower rim of the nares and the cribriform plate ($p < 0.001$) were significantly longer in males (Figure 3(A),(B)).

In all specimens the ala nasi had to be slightly lifted or at least touched by the shaft of the probe when advancing its tip along the palate. In 20 specimens advancement along the palate required an intense elevation of the ala nasi (Figure 4(C),(D)). In these specimens, entering the pharynx through the uppermost part of the choana was possible by only slightly elevating the ala nasi with the shaft of the probe (Figure 4(A),(B)).

4 | DISCUSSION

Excellent descriptions of the anatomy of the nasal cavity and nasopharynx are provided in a plethora of scientific publications and

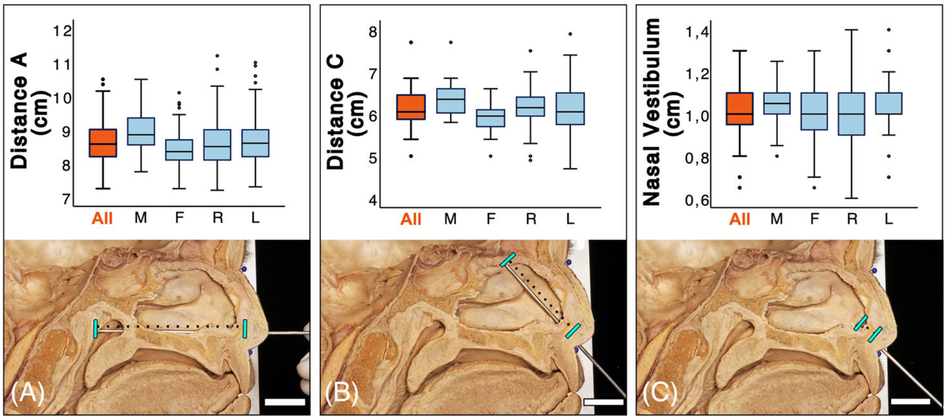

FIGURE 3 Distances. (A)–(C) Distances between lower rim of nares and posterior wall of nasopharynx (A), cribriform plate (B), and border of vestibulum nasi (C). Scale bars, 2 cm

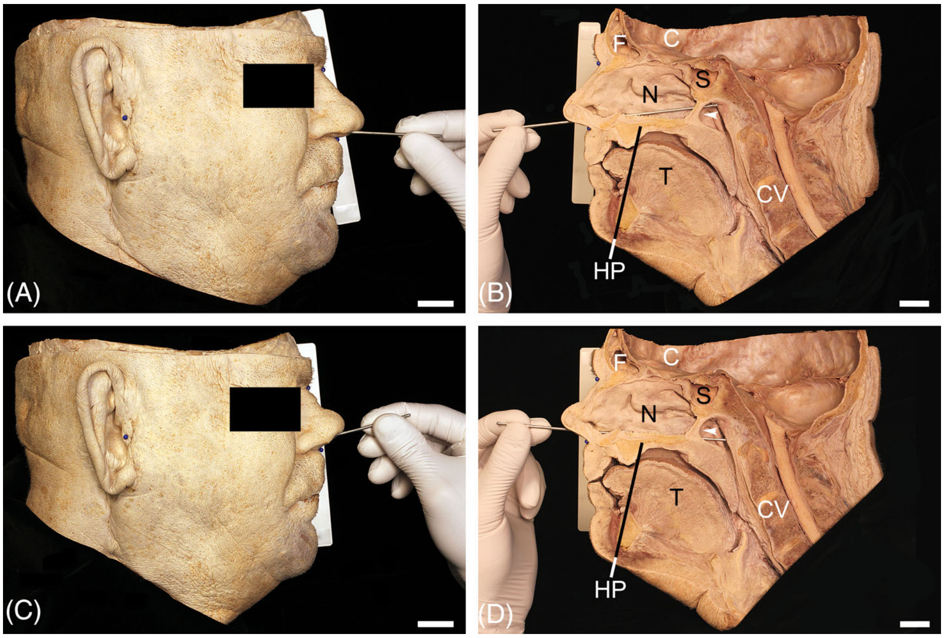

FIGURE 4 Required elevation of ala nasi. (A, B) Probe inserted as directed toward the external acoustic meatus. It touches the ala nasi and passes through the uppermost part of the choana (arrowhead) into the fornix pharyngis. (C, D) Probe inserted in the suggested direction along the hard palate. Note the very intense elevation of the ala nasi. C, anterior cranial fossa; N, nasal cavity; HP, hard palate; F, frontal bone; S, sphenoid bone; T, tongue; CV, bodies of cervical vertebrae. Scale bars, 2 cm

textbooks (Drake et al., 2015; Logan et al., 2016; Netter, 2019; Sobotta et al., 2008). Also, a large number of tutorials advising the performance of nasopharyngeal swabs are available (CDC, 2020; Falcone, 2020; Marty et al., 2020; NMC, 2020; Pondaven-Letourmy et al., 2020). Despite this, swabs of nasopharyngeal test kits are often inserted in weird angles. In such cases, it is to be expected that the tip of the swab does not penetrate the choana and a nasal swab is performed, which collects material from the mucosa of the nasal cavity.

This might be sufficient for a small spectrum of pathologic agents (Rawlings et al., 2013; Ylikoski et al., 1989). Yet, it is less sufficient than collecting nasopharyngeal material for pathogens having a high affinity to pharynx mucosa, such as SARS-CoV-2, Epstein–Barr-Virus, and many others (Babady et al., 2018; Yao et al., 1985). The sensitivity in diagnosing such pathogens is higher if material is collected from the oro- or nasopharynx (Chen et al., 2020; Heikkinen et al., 2002; LeBlanc et al., 2020; Péré et al., 2020; Vlek et al., 2021). It is therefore of utmost importance to gain precise knowledge of the anatomy of the upper airways to correctly and unquestionably collect pharynx material by means of nasopharyngeal swabs. Our simulations of 314 nasopharyngeal swabs provide valuable parameters, such as angles and distances in respect to easily identifiable anatomical landmarks and orienting lines. As the most important one we consider the line between nasion und subnasale, since it defines a near coronal plane that is perceived as a "plane representing the face". Of almost equal importance is the often-suggested line between subnasale and tragus, which provides a fairly good estimation of hard palate orientation from outside.

However, as our data reveal, suggestions to use the tragus as a landmark for estimating the direction to advance swabs (Marty et al., 2020), although highly valuable, are only half the message. Our simulations demonstrate that advancement of a swab along the subnasale/tragus line merely in 44.1% succeeds in entering the pharynx. Advancing a swab, while keeping strict contact with the hard palate directs it in an angle of up to 18° (95% interval: 3°–17°) below this line. Hence advancing the swab in an angle of 18° would always be successful in directing its tip into the lower parts of the nasopharynx. However, our measurements also show that advancement in an angle of 14° is acceptable as well, since it permits at least penetration through the uppermost parts of the choana toward the fornix pharyngis. Therefore, if using the subnasale/tragus line as a guide, we suggest to advance the swab in an angle of 14° below this line until its tip touches the hard palate and then use the hard palate to guide further advancement.

We therefore conclude that, independent from anatomical landmarks and orientation lines, the most secure way to successfully perform nasopharyngeal swabs is to advance the swab by keeping constant contact with the hard palate and, ideally, the adjacent septum nasi. In all examined specimens this required pushing the lower rim of the ala nasi slightly upwards with the shaft of the swab. Thus, upward movement of the ala nasi is a simple and easily visible sign for correct swab advancement.

As a result of our measurements and analyses, our study now provides three essential lessons for correctly and successfully performing nasopharyngeal swabs (Figure 5):

1. Start with inserting the swab in a steep upward angle for approximately 1 cm into the nares.
2. Push the shaft of the swab upwards, while further advancing, until the tip touches the hard palate and the ala nasi is slightly elevated.
3. Advance the swab by constantly keeping contact with the hard palate until feeling the resistance of the backside wall of the nasopharynx. As an additional visual control, keep sure that the ala nasi is slightly elevated and advance as if targeting an area below the tragus.

In 7 of the 314 nasopharyngeal swab simulations, we were unable to advance the probe toward the choana, because of protrusions from the hard palate, which effectively blocked the way along the lower nasal meatus. In 2 head/neck specimens this was bilaterally. In those individuals performing nasopharyngeal swabs would have been entirely impossible.

Sceptics of Covid-19 tests quite often argue that during the collection of material from the nasopharynx or nasal cavity, the tips of swabs might end up inside the cranial cavity and destroy basal parts of the forebrain (Sullivan et al., 2020). The cribriform plate is blamed as potential entry point. Although the cribriform plate is a very thin osseous structure (Figure 1(H)), we consider it as extremely unlikely to penetrate it with commercial kits for nasopharyngeal swabs. Nevertheless, we decided to make use of the half specimens for researching how a swab has to be advanced to put the cribriform plate in danger. As expected the angle in respect to the subnasale/nasion line is rather steep, and it is similar to the angle in which we suggest the swab should be inserted into the nose for 1 cm when starting a nasopharyngeal swab. Yet, we consider it extremely unlikely that educated

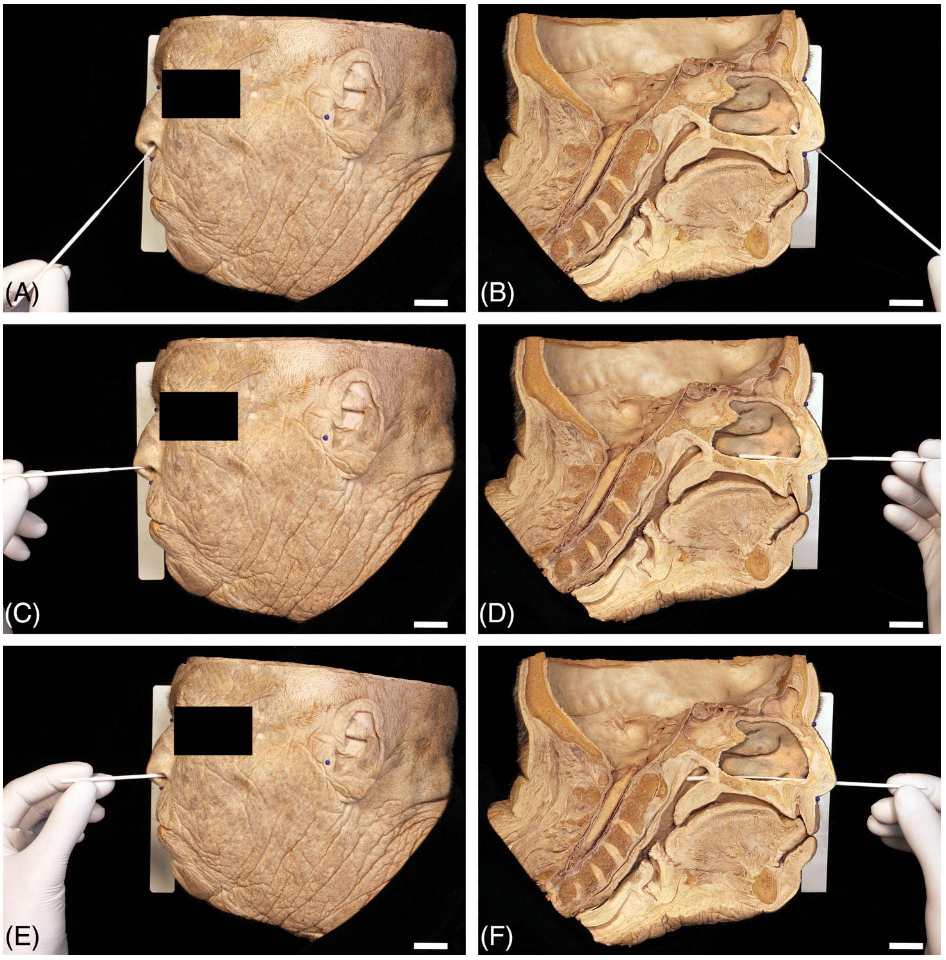

FIGURE 5 Simulation of a secure and successful nasopharyngeal swab in a mediansagittally sectioned head/neck specimen. (A, B) Swab inserted 1.1 cm following the soft tissue cushion laterally to the subnasale. (C, D) Elevated and advanced swab touching the hard palate with its tip. Note the relation of shaft and ala nasi. (E, F) Swab advanced through the lower rim of choana and touching the posterior wall of nasopharynx. Scale bars, 2 cm

personnel will try to advance the swab in such an angle and, if such an angle is accidentally used, it is highly unlikely that the swab is pushed strongly enough to penetrate the cribriform plate. It is more likely that in such a case the swab causes an irritation of the olfactory mucosa and the olfactory nerve, which might result in temporary reduction of olfaction.

Our study focuses on simulating nasopharyngeal swabs and defining important anatomical parameters. However, our results also have strong impact on collecting material from the nasal cavity. Since it is considered that also non-medicals and children can perform nasal swabs, they recently became trendy for self-testing for SARS-CoV-2 infections (Péré et al., 2020). The swabs are suggested to be inserted in a steep upward angle (BMBWF, 2021), which roughly directs toward the cribriform plate. Therefore, the danger for the cribriform plate and the olfactory mucosa is higher when performing nasal swabs compared to performing nasopharyngeal swabs. Our measurements suggest that swabs of nasal testing kits should not be inserted for a distance longer than 3–4 cm into the nasal cavity of adults to avoid temporary reduction of olfaction.

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