

Assessment of Aerosol Generating Events During the Nasopharyngeal Swabbing for SARS-CoV-2 Detection

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Research Article

Keywords: nasopharyngeal swab, infectious aerosols, viral load, COVID-19, SARS-CoV-2

Posted Date: September 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-775996/v1>

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Abstract

Background: A variety of medical procedures are classified as aerosol generating. However there is no consensus on whether some procedures such as nasopharyngeal swabbing can generate aerosols. During specimen collection, the contact of the nasopharyngeal swab with the respiratory mucosa often triggers defense reflexes such as sneezing and coughing, which generate airborne particles. The accumulation and persistence of a viral load from infectious aerosols for hours after their generation can represent a threat for increased spread of infection.

Methods: Prospective observational cohort study in individuals tested for RT-PCR SARS-CoV-2 from July to October 2020. Participants were evaluated for the occurrence of aerosol generating events (AGEs) triggered by the nasopharyngeal swabbing. We used descriptive statistics to analyze the data set and the chi-square test for AGE comparison between sexes. **Results:** Among 1239 individuals, we reported 264 in which AGEs were triggered by the specimen collection. 97 individuals tested positive for SARS-CoV-2, of which 20 presented AGEs. There were no significant differences in the occurrence of AGEs by age, but significant differences have been identified between sex and the occurrence of AGEs both in the SARS-CoV-2 negative and SARS-CoV-2 positive individuals.

Conclusions: The risk of coughing or sneezing triggered by the nasopharyngeal swabbing was high among tested individuals. Testing facilities should ensure adequate availability of personal protective equipment (PPE) for the testing personnel, ensure appropriate ventilation of the rooms, and develop additional strategies to limit the risk of contamination of other participants to the testing session from potentially infectious and persistent aerosols.

Introduction

Sneezing, coughing, and even higher amplitude breathing can produce natural water droplets which can enclose a variety of particles from epithelial and immune cells to different infectious agents such as bacteria, fungi and even viruses.[1] When the aerodynamic size of a viral particle becomes appropriate, it emerges as an airborne virus which can infect other people or contaminate surfaces through the ambient air from even the simplest daily habits.[2] Droplets $\leq 5\mu\text{m}$ in diameter become airborne and such aerosols can linger in the ambient air and remain pathogenic for many hours after being produced. SARS-CoV-2 was reported to be twice more aerostable than the influenza virus and 4 times more aerostable than filoviruses.[3] The place where the aerosols are generated can dictate whether sufficient viral copies are passed from one host to another to become pathogenic. While outdoor spaces can lead to the dispersion of the viral copies, enclosed spaces can hold up an increased viral load in the air for prolonged periods of time after the occurrence of an aerosol generating event (AGE). This can become a concern in specimen collection units for SARS-CoV-2 testing since the contact of foreign objects (e.g. a flocked swab) with the mucosa of the respiratory tract of a person being tested can trigger defense reflexes such as coughing and sneezing. Addressing the potentially infectious and persistent aerosols resulting from such events prompts for immediate protective measures to be taken in order to prevent the contamination of testing

personnel and the following participants to testing. COVID-19 is believed to be transmitted through droplets and aerosols generated when an infected person coughs, sneezes or even talks or exhales. The fate of the droplets is mostly determined by the droplet size as the most important factor affecting their dispersion and deposition on surfaces.[2] Larger droplets will deposit near the emission point, while smaller droplets evaporate faster in the form of aerosols (< 5µm in diameter) and linger in the air, drifting and traveling meters or tens of meters in indoor air.[4] **(Fig. 1)** Aerosolization can occur when hosts spread the virus directly in the ambient air (primary aerosolization), while secondary aerosolization can occur when the viruses are dispersed by air displacements or movements from surfaces.[5]

The highly contagious SARS-CoV-2 has already spread rapidly on a global scale claiming 2000 times more lives than the previous SARS and MERS coronavirus epidemics combined.[6] The increased capacity of infecting many people at a time is believed to be attributed to the capability of becoming trapped in infectious droplets and aerosols which plays a vital role in contracting susceptible nearby hosts.[7] Although the pathogenic viral load necessary to infect a host is not clear, scientists speculate that a few hundred copies of SARS-CoV-2 can be enough to cause COVID-19 in susceptible persons.[8] This raises concerns about the potential persistence in the air, contamination of objects and human-to-human transmission of the virus through the accumulation of a persistent viral load from infectious aerosols in specimen collection rooms. Infectivity assessments with SARS-CoV-2 have reported the virus to remain viable and pathogenic for hours in the ambient air and for days on surfaces after aerosolization.[9]

The PCR analysis of a nasopharyngeal swab sample is currently the gold standard for COVID-19 testing. [10] The nasopharyngeal mucosa of the respiratory tract is one of the most exposed areas to airborne pathogens, and is the site with the highest viral replication in SARS-CoV-2 infection, with no distinct differences in viral loads or detection rates between nasopharyngeal and oropharyngeal swabs.[11]

The flocked swab specimen collection allows for the coverage of a wide area to be sampled, as it can run from the nasal cavity all the way through the nasopharynx and back to collect mucosal cells on its surface.

While sampling is not traumatic itself, the contact of the respiratory mucosa with the flocked swab can initiate defense reflexes such as sneezing, coughing and lacrimation. While lacrimation poses little risks for contamination,[12] AGEs such as sneezing and coughing can generate airborne viral particles which may persist for long periods in the ambient air and pose a risk for the examiners and the following participants to testing. These risks can comprise both the infection of other participants and the subsequent contamination of the samples from the potential aerial contamination of the flocked swabs during handling. This raises concerns about the safety of specimen sampling rooms and stresses the need to implement protective measures to avoid the infection of other participants to testing and the contamination of specimens from the exposure of the flocked swab and opened tube to ambient air during the sampling.

Methods

Study design and participants

We performed a prospective observational cohort study between June 2020 and October 2020, evaluating the occurrence of AGEs such as coughing and sneezing during the nasopharyngeal swabbing for RT-PCR SARS-CoV-2 testing. At enrolment, participants consented to use of information for research and agreed to applicable privacy policies and terms of use. Our study was approved by the Ethics Committee of the University of Medicine and Pharmacy “Iuliu Hațieganu” Cluj-Napoca (No. 16761/09.06.2020).

Procedures

Participants were observed during the nasopharyngeal swabbing for the occurrence of AGEs such as coughing and/or sneezing triggered during the sampling. This was recorded in the database together with the demographical characteristics. COPAN's® flocked swabs were used for the nasopharyngeal specimen collection which was collected by a certified physician (CCA) for all the samples. The specimens were collected using the method described by Francisco M et al 2020 [13] but with supplementary protective measures for both participants and examiners consisting in using PPE by the personnel and resorting to keeping the facemasks over the mouth of participants during specimen collection to limit aerosol spreading, together with good ventilation of the sampling room, decontamination of air, walls and objects with combined physical and chemical agents after each participant.

Statistical analysis

All data collected during nasopharyngeal swabbing were introduced into an Excel file which was then exported into SPSS. For descriptive statistics we used: mean, standard and median deviation for the continuous data and for the ordinal or nominal data the frequencies and percentages were used. For AGE comparison between both sexes we used chi-square test. SPSS version 21 was used for all the statistical analysis considering a significant P value of < 0.01 .

Results

Between July 1 and October 30, 2020, we enrolled 1239 participants to our study, 583 males and 656 females, with ages ranging from 1 to 89 and a median age of 38. The prevalence of AGEs triggered by the nasopharyngeal swabbing in our study was 21.23%, consisting of coughing in 13.96% and sneezing in 8.56% of participants (Fig. 2). The occurrence of AGEs was higher in male (26.24%) than in female participants (16.77%) (Fig. 4). The baseline characteristics of the study participants are presented in Table 1.

Table 1
Baseline characteristics in all individuals compared with individuals presenting AGEs in the study group.

		Total individuals	individuals with AGEs
Sex	M	47,05%	12,35%
	F	52,95%	8,96%
age, years	< 25	10,25%	2,34%
	25–34	25,59%	5,73%
	35–44	29,06%	6,46%
	45–54	18,40%	3,39%
	55–64	12,75%	2,82%
	≥ 65	3,95%	0,56%
	SARS-CoV-2	positive	7,83%
	negative	92,17%	19,61%

In this cohort we recorded 97 positive participants in the RT-PCR SARS-CoV2 analysis, 53 males and 44 females. The prevalence of triggered AGEs among the positive participants was 20.61% consisting of coughing 15.46% and sneezing in 5.15% (Fig. 3). Out of the 20 positive participants which presented AGEs, 14 (70%) were male and 6 (30%) were female.

There were no significant differences between the age group (< 25, 25–34, 35–44, 45–54; 55–64; >65) and the occurrence of coughing and sneezing triggered by the nasopharyngeal swabbing ($p = 0.666$).

There were no significant differences between SARS-CoV-2 positive and SARS-CoV-2 negative participants in the occurrence of coughing and sneezing ($p = 0.763$).

Comparing the two sexes, the prevalence of AGEs in men was higher than in women during the nasopharyngeal swabbing ($p < 0.01$), both in the SARS-CoV-2 positive and SARS-CoV-2 negative groups. (Fig. 4, 5)

Discussion

Our results show that the nasopharyngeal swab specimen collection is associated with a high occurrence of defense reflexes of sneezing and coughing triggered by the contact of the nasopharyngeal mucosa with the swab. Here we have shown that AGEs have occurred in both uninfected and infected persons, being triggered in more than 1 in 5 participants to nasopharyngeal swabbing. This can become a concern when many infected participants show up to testing as it creates the conditions for airborne viral spreading. AGEs have the potential of contaminating the ambient air and the surrounding surfaces,

posing potential contamination risks for the following participants to testing and also for the following collected specimens.

As effective decontamination procedures can be easily applied on the empty specimen collecting room, this can become controversial when decontamination is required in between participants in which AGEs such as reflexes of sneezing and coughing have occurred. Here, the use of an UV light lamp is impractical as it requires eye and skin protection for the room occupants and a prolonged period of exposure for efficacy, while applying disinfectants with the participants inside can be a delicate subject. However, safer alternatives could be used to limit the risk of infecting other participants and contaminating subsequent samples after the occurrence of an AGE.

STRATEGIES OF LOWERING THE ODDS OF CONTAMINATION FROM AN AGE

Providing adequate ventilation and volume of air

Providing adequate ventilation to the sampling room could ensure that the viral load in the ambient air will stay at low levels from air exchanges with the exterior. A natural ventilation is preferred against mechanical ventilation systems since air recirculation devices can accommodate and potentially mobilize viral particles in the air from exhaust outlets.[14] In cold weather, the air exchanges with the exterior through open windows may ensure a higher rate of air replacement through inside-outside temperature differences contributing to a faster clearance of the viral load in the ambient air of the warmer testing room.[15] The total volume of air in the sampling room is of equal importance, since aerosols tend to become dispersed in the total volume of air. This is why the bigger the sampling room, the more increased the total volume of air will be, leading to a more reduced concentration of the viral load in the ambient air after the occurrence of an AGE. Therefore, the ideal sampling room with regards to an adequate volume of air will be the biggest room in the building with as little furniture as possible and many windows for natural ventilation. This is to prevent that more than several hundred persistent copies of the virus will linger in the air in the same place at the same time to potentially become pathogenic.[8] Waiting rooms should be replaced by a rigorous appointment schedule to prevent cramming of participants inside indoor places. A 5 minute interval between participants in our study was enough to allow the necessary time for specimen collection and decontamination while preventing the cramming of participants. If a waiting room is however indispensable, it should respect distancing and have adequate natural ventilation.

Using washable surfaces

A decontamination of surfaces should be achieved after the occurrence of an AGE before the next participant is invited in. However, this can be done if the sampling room is equipped with readily

washable surfaces of tables chairs and walls, on which the decontaminants can be effectively applied. Since airborne viruses can become embedded in fabrics, the textile covering of chairs, table or windows should be avoided. Moreover, textile walls such as the ones used in military tents could accommodate viral particles after an AGE and an on-site decontamination would be impractical. Besides, such particles embedded in the fabric could be mobilized by weather conditions such as strong winds, heavy rain or hail and become airborne again. This is why containers should be preferred against military tents to provide proper cleaning conditions for the decontamination of walls and surfaces after the occurrence of an AGE.

Keeping the facemasks over participant's mouth

The facemask of participants becomes of vital importance as it can limit both spreading and contacting the virus. While wearing the facemask should be compulsory in the sampling rooms, its removal during the specimen collection procedure becomes necessary. However, the partial removal from the nose area while still covering the mouth can facilitate specimen collection by nasopharyngeal swab sampling, limiting viral spread through a potential AGE, while also limiting the inhalation of potential pathogenic viral aerosols lingering in the ambient air by advising the person being tested to only breath by the mask covered mouth during the specimen collection (fig.6) A lateral positioning of the examiner during sampling could potentially avoid facing the direct current of expelled infectious particles by AGEs to a greater extent than a face-to face positioning. Since the risk of contamination among frontline healthcare workers wearing a PPE is 3-fold higher than in the general population,[16] keeping the facemasks of participant on their mouths during the nasopharyngeal swabbing and standing sideways from the direction of potential infectious particles expelled by an AGE during the nasopharyngeal swabbing could limit the contamination of the examiner and the surroundings.

Using disinfectants after an AGE

The use of chemical disinfectants or a UV lamp after an AGE can limit the contamination of the surrounding objects and the air. However, there are not many disinfectants that can be safely applied on objects and air with the participants inside, as their contact with the skin, eyes and respiratory mucosa can be harmful. This is why their use is impractical in sampling rooms, as they require the removal of all participants, a prolonged exposure time, and the proper ventilation to prevent inhalation and irritation of the respiratory tract mucosa and eyes. 70% ethylic alcohol is an effective agent against enveloped viral pathogens as it acts on the integrity of the lipid bilayer membrane of the envelope while also being relatively safe for human exposure with the least harmful effects. However, using only alcohol can leave traces of leftover intact viral genome to contaminate surfaces and test positive to the PCR analyses even if being nonpathogenic. To address this limitation, 70% ethylic alcohol can be potentiated with an adjuvant that induces structural breaks in the viral genome. Methylene blue (MB) can be used as such an adjuvant since it is safe for human use at low concentrations and it acts as a photosensitizer in association with visible light.[17] [18] This induces the generation of multiple reactive oxygen species

(ROS) with singlet oxygen being the most abundant, leading to depurination, guanosine oxidation and strand cross-linking, with consequent viral genome degradation.[19] MB photooxidation was shown to be effective against multiple viral pathogens[17] including SARS-CoV2.[20] While alcohol can facilitate the MB passage through the viral envelope, this is done even in the absence of alcohol by the natural property of MB to cross lipid bilayer membranes and this effect is being exploited in photoinactivation procedures of the plasma and vital staining of cellular organelles being a safe agent for human use.[21] The combined effect of alcohol and MB could address both viral pathogenicity and the risk of positive results from leftover integral viral genome and could be used in the form of spraying or steaming the air and objects after an AGE to address airborne viral particles. Such a combination is available in blue rubbing alcohol formulations. Ammonium salts in commercially available biocide products can be used as alternatives to ethylic alcohol for the decontamination of the microaerophore, and could potentially be used in the presence of other participants wearing facemasks. Good laboratory practices such as limiting the exposure time of the nasopharyngeal swab and the opened collection tube and its cap in the ambient air could further reduce the risk of contamination of the swab from the air or the influence of decontaminants contained in the air on the results of testing.

Using the relative humidity of air after an AGE

Relative humidity (RH) of air is one of the most important factors affecting airborne virus infectivity as a direct link between the persistence, survival and pathogenesis of viral particles and RH was described.[5] Infectivity studies show that low RH tends to conserve the infectivity of enveloped viruses, while nonenveloped viruses become more stable at high RH.[22] It was shown that high RH is deleterious to the survival of the aerosolized enveloped coronaviruses SARS-CoV-2[3], MERS-CoV[23] and HCoV-229E.[24] Aerosolized particles tend to bind to other fluid molecules in the air by electrostatic attraction, impaction, interception and diffusion[2] so an increased size and weight of viral aerosols will lead to a faster settling rate and a decreased inhalability. Using methods of increasing the RH of the ambient air could reduce the risk of infection after an AGE, while influencing the chemical composition of water particles using adjuvants to neutralize virions could address the airborne virions after an AGE. This could be obtained by exploiting the viral photoinactivation potential of MB [17] [25] and the envelope damaging effect of ethylic alcohol together with the faster sedimentation effect induced by increased RH. Spraying or steaming MB potentiated water or alcohol in the ambient air immediately after an AGE could be an acceptable method of increasing viral sedimentation rate and the viral photoinactivation in the air and on the ground and surfaces. Moreover, adding the effect of heat to such system could increase the effect of damaging the integrity of the viral envelope. Increasing the relative humidity and temperature gas been shown to accelerate SARS-CoV-2 inactivation on surfaces. [26] Since the new coronavirus is shown to present a high degree resistance to physical aggressions such as mechanical forces and heat,[27] the use of a combined decontamination strategy which may include chemical (alcohol, MB) and physical agents (heat, RH, photoinactivation) becomes justified.

Conclusions

The highly contagious SARS-CoV-2 persistent airborne viral aerosols produced by an infected person through sneezing and coughing represent a real threat to both the present room occupants or new occupants over the following hours from their emission time. The nasopharyngeal swab sample collection triggers AGEs in 1 in 5 participants to testing as our study shows. This indicates that it is only a matter of chance until AGEs occur in many infected participants during a nasopharyngeal swab specimen collection session. Given the ever increasing number of infected persons, the chance of having AGEs from infected participants grows as the number of positive cases increases. As testing requires the removal of the facemask in the collection room this becomes a potential threat to the following participants to specimen collection as viral aerosols produced by sneezing, coughing, speaking or even exhaling can linger for many hours in the ambient air contributing to the viral load of the room air. However, reducing the chances of contamination could be achieved using a well-designed specimen collection room with plenty of natural ventilation and light. Alternatively, collection of specimens in an outside non-fabric tent or through a drive-through approach are also possible alternatives. In addition, readily washable surfaces and an available sprayer or steamer with a harmless decontaminant for humans but potent antiviral activity should be used. Since the SARS-CoV-2 shows high resistance to multiple types of aggressions, the use of a combined strategy using both physical (heat, light, RH) and chemical (alcohol, MB photosensitizer) becomes justified. 70% ethylic alcohol enriched with MB applied as a hot steam in ambient air and on objects after the occurrence of an AGE can represent a rapid and safe method of decontamination in between participants. Requesting participants to keep their facemasks upon entry and only removing it from the nose area as the nasopharyngeal swab specimen is being collected while breathing only through the mouth, could limit both exposure to preexistent airborne viral particles and their generation by AGEs. A similar stepwise approach should be implemented in all testing facilities when dealing with various types of enveloped viral pathogens with airborne transmission. Our study suggests that adequate PPE should be ensured for the testing personnel, while the development of additional strategies to limit the risk of contamination of other participants to the testing session from potentially infectious persistent aerosols should be implemented. Our findings represent arguments for implementing such measures and could contribute to a practice change in the current sampling method for the detection by nasopharyngeal swabbing of SARS-CoV-2 and other enveloped viral pathogens with airborne transmission.

Declarations

Funding.

No funding was received for conducting this study.

Conflicts of interest/Competing interests.

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability.

Not applicable

Ethics approval.

This is an observational non-interventional study. All methods were performed in accordance with the relevant guidelines and regulations. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hațieganu" Cluj-Napoca (No. 16761/09.06.2020).

Authors' contributions.

CCA performed the study concept and design. CCA, CS and NA performed the sampling and acquisition of data. CGL performed the data analysis. All authors contributed to interpretation of data and revision of the report. BNI revised the initial manuscript and supervised the study. NMG critically revised the final manuscript.

Consent to participate.

Verbal informed consent was obtained prior to performing the data acquisition to limit the exposure period of participants to the testing facility's environment. Due to the observational non-interventional nature of the study and being a public health issue, a written informed consent was unnecessary according to national regulations. (Lege nr. 677/21.11.2001, art.9a, available at <http://legislatie.just.ro/Public/DetaliiDocument/32733>). The study methods have been performed in accordance with the Declaration of Helsinki.

Consent for publication.

Not applicable.

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Figures



Figure 1

Schematic representation of the generation of Flügge's droplets by AGEs. Specimen collection by nasopharyngeal swabbing can generate reflexes of coughing and sneezing. In an infected person, such airborne particle producing events can lead to virus containing droplets (Flügge's droplets) falling in the proximity of the infected person while some droplets become aerosols by evaporation into droplet nuclei which can linger for many hours in the ambient air of enclosed spaces.

Prevalence of AEGs in total individuals



Figure 2

Distribution of AEGs in the study group.

Prevalence of AEGs in SARS-CoV-2 positive individuals

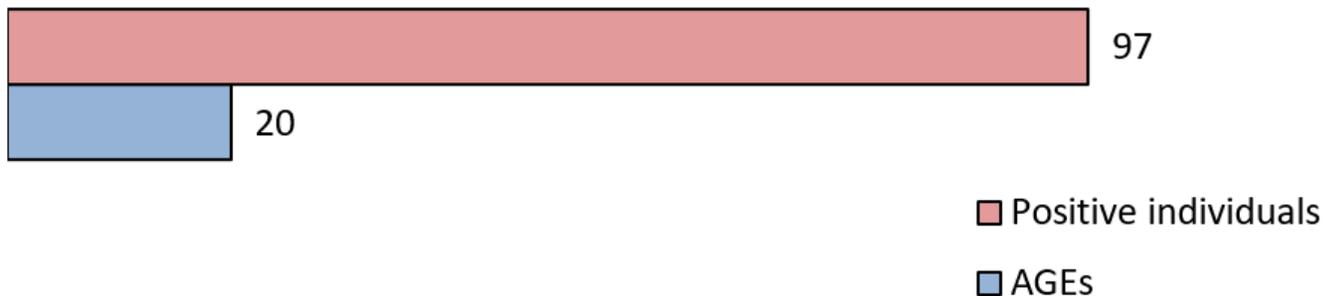


Figure 3

Distribution of AEGs in SARS-CoV-2 positive individuals.

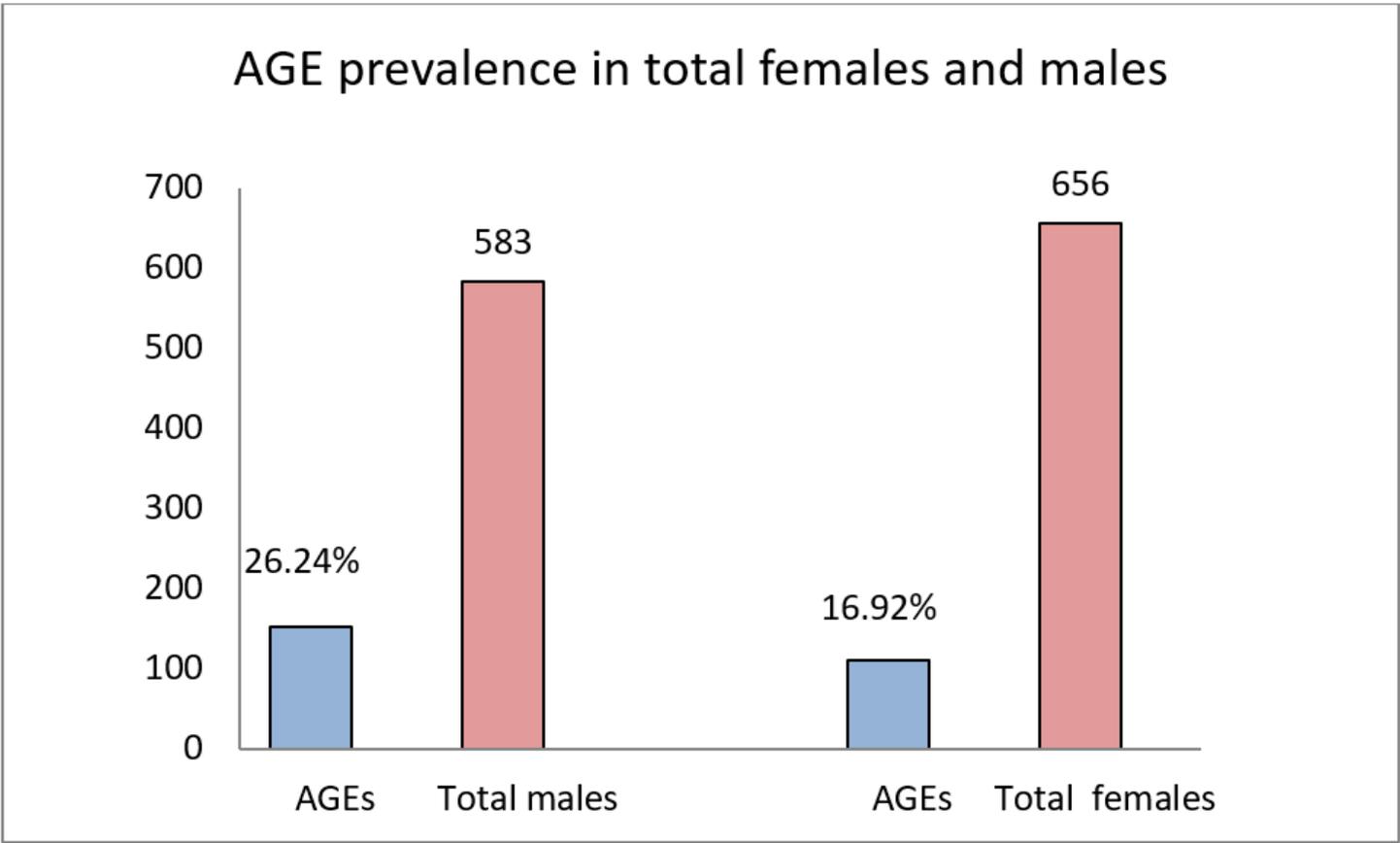


Figure 4

Distribution of AGEs by sex in all individuals.

Prevalence of AGEs in positive males and females

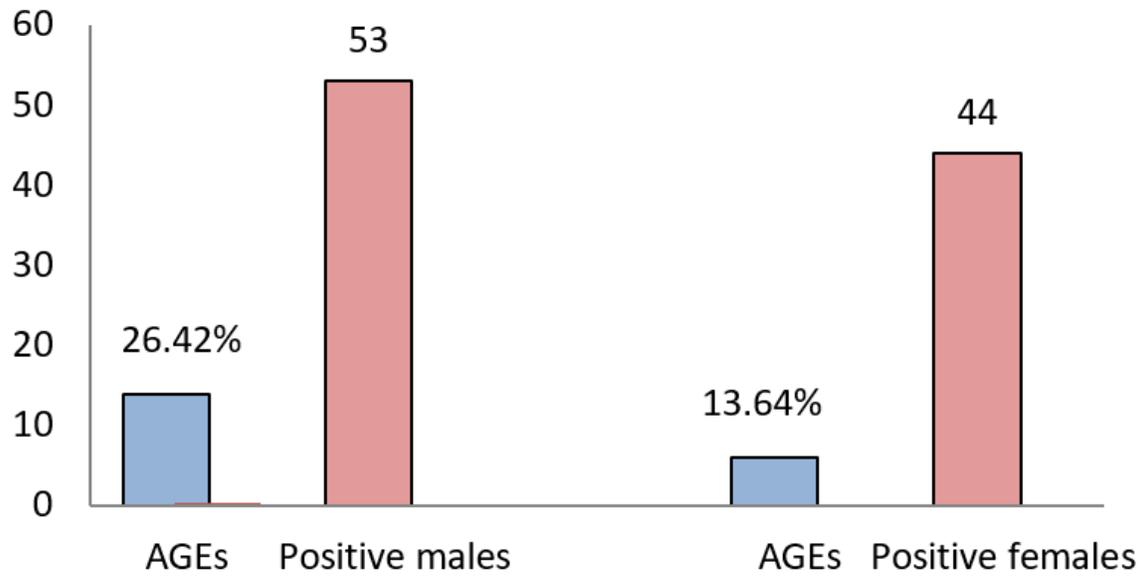


Figure 5

Distribution of AGEs by sex in SARS-CoV-2 positive individuals.

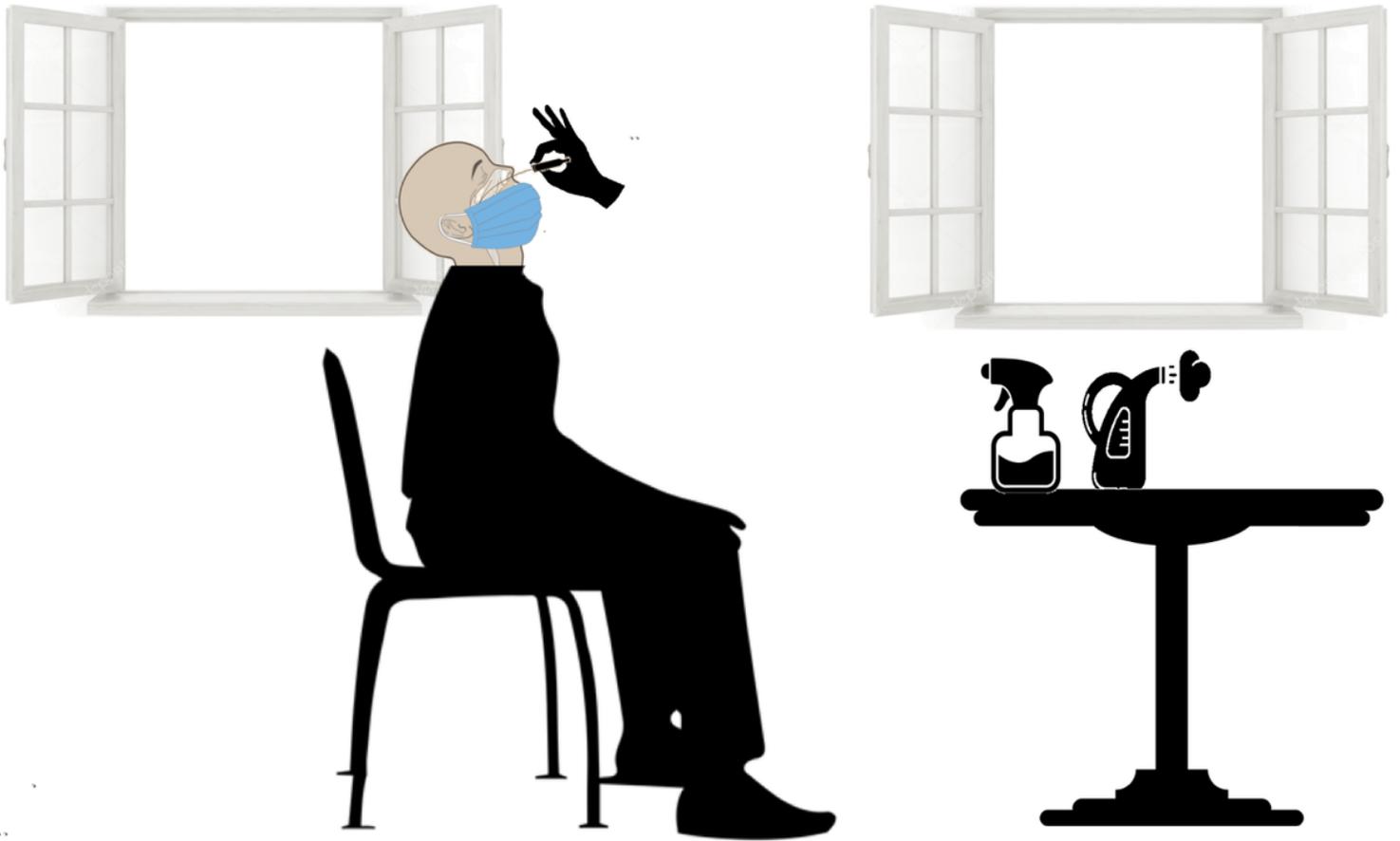


Figure 6

Proper sampling conditions for COVID-19 by nasopharyngeal swabs should provide good ventilation and light, washable surfaces and walls, readily available and safe decontaminants for ambient air and surfaces in the form of sprayer or steamer, while participants should be advised to keep their masks on their faces and only removing it from the nose area during the specimen collection to limit viral spreading by coughing or sneezing and to prevent breathing in aerosolized viral particles by only breathing through their mouth during the procedure.