Differentiation of aspirated nasal air from room air using analysis with a differential mobility spectrometry-based electronic nose: a proof-of-concept study

Jussi Virtanen[1,2], Anna Anttalainen[3], Jaakko Ormiskangas[2,4], Markus Karjalainen[2,3], Anton Kontunen[2,3], Markus Rautiainen[1,2], Niku Oksala[2,3,5], Ilkka Kivekäs[1,2] Antti Roine[3,6]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Tampere University Hospital, Finland

[2]Faculty of Medicine and Health Technology, Tampere University, Finland

[3]Olfactomics Ltd, Tampere, Finland

[4]Faculty of Engineering and Natural Sciences, Automation Technology and Mechanical Engineering Unit, Tampere University, Finland

[5]Vascular Centre, Tampere University Hospital, Finland

[6]Department of Surgery, Tampere University Hospital, Hatanpää Hospital, Tampere, Finland

Abstract

Over the last few decades, breath analysis using electronic nose technology has become a topic of intense research, as it is both non-invasive and painless, and is suitable for point-of-care use. To date, however, only a few studies have examined nasal air. As the air in the oral cavity and the lungs differs from the air in the nasal cavity, it is unknown whether aspirated nasal air could be exploited with electronic nose technology. Compared to traditional electronic noses, differential mobility spectrometry uses an alternating electrical field to discriminate the different molecules of gas mixtures, providing analogous information. This study reports the collection of nasal air by aspiration and the subsequent analysis of the collected air using a differential mobility spectrometer. We collected nasal air from ten volunteers into breath collecting bags and compared them to bags of room air and the air aspirated through the device. Distance and dissimilarity metrics between the sample types were calculated and statistical significance evaluated with Kolmogorov-Smirnov test. After leave-one-day-out cross-validation, a shrinkage linear discriminant classifier was able to correctly classify 100% of the samples. The nasal air differed ($p < 0.05$) from the other sample types. The results show the feasibility of collecting nasal air by aspiration and subsequent analysis using differential mobility spectrometry, and thus increases the potential of the method to be used in disease detection studies.

1. Introduction

Volatile organic compounds (VOC) are potential biomarkers of diseases, and their use in disease diagnostics has become a fast-growing field of research [1, 2]. A sample of exhaled human breath usually contains between 200 and 300 VOCs [3, 4]. Moreover, the sampling of exhaled breath is a non-invasive, painless technique that has potential for point-of-care use.

The gold standard method for VOC analysis is gas chromatography-mass spectrometry (GC-MS), which can identify individual compounds in breath samples. However, the method is expensive and requires experienced personnel to operate it. An additional drawback of mass spectrometry is that it often requires selective sampling, which limits the breadth of the molecules analyzed. In contrast to the GC-MS sampling method, the electronic nose (eNose) analyzes VOCs qualitatively, typically using an array of sensors that deliver a measurement signature, which could represent the VOC pattern of a certain disease [2]. A pattern recognition algorithm is then taught to discriminate different VOC patterns, and thus potentially discriminate diseased patients from healthy ones. eNose devices are usually relatively compact and they can perform a sample analysis in minutes. Indeed, eNose technology has even been shown to outperform mass spectrometry.

Differential ion mobility spectrometry (DMS) - also known as field asymmetric ion mobility spectrometry (FAIMS) - is a technique that uses an alternating electrical field to discriminate the different molecules of a sample. Although DMS is not based on sensor arrays, as is the case with traditional eNoses, it provides analogous information on gas-phase molecules. The operating principle of DMS makes it less prone to drifting and the batch-to-batch variation that has plagued many semiconductor sensor-based eNoses [5]. In DMS, however, the molecules of the

sample need to be ionized. Although different ionization methods exist, not all molecules can be ionized using one specific method. Therefore, DMS is selective to a certain range of VOCs. Theoretical advantages of DMS over microscale FAIMS are the longer residence time and the higher number of oscillations, which improves the separation capacity of the system. A further theoretical advantage of DMS over drift and travelling waves is the ability to perform continuous analysis compared to the pulsed measurements of the other methods. The additional advantage of DMS and FAIMS is that the method provides information on the behavior of molecules in high and low fields [6].

Numerous studies have examined the application of eNose technology in disease diagnostics with encouraging results. Classic asthma, for example, has been distinguished from chronic obstructive pulmonary disease with a sensitivity of 91% and a specificity of 90% [7]. Further, an ion mobility spectrometry-based eNose was able to diagnose prostate cancer from urine with a sensitivity of 78% and a specificity of 67% [8]. Using DMS analysis of urine samples, malignant ovarian tumors were differentiated from healthy controls with a sensitivity of 91% and a specificity of 63% [9]. As reported in a review by Farraia *et al.* [10], many published studies use exhaled breath as a sample material. We are, however, aware of only a few studies that have investigated the use of nasal air with an eNose. In these studies, patients exhaled through the nose, or the air was aspirated during normal respiration either into a breath-collecting bag or into an eNose [11-14]. Thus, air from the lungs and the pharynx could have affected the results since it is known that expiratory flow rate, breath hold and the fraction of breath analyzed can alter the measurement signature of the eNose [15, 16] and have a subsequent impact on the reproducibility of the measurements.

To only examine nasal air, the sample should be collected using aspiration as described by the American Thoracic Society and the European Respiratory Society (ATS/ERS) in their guidelines for the measurement of nitric oxide (NO) [17]. The

aim of this study is therefore to report the collection and subsequent analysis of nasal air using an electronic nose based on DMS technology.

2. Materials and methods

2.1 System for the aspiration of nasal air

The device used for the aspiration of nasal air was a suction pump SP 625 EC-LC-DU (Spiggle & Theis Medizintechnik GmbH, Germany) operated with AA-batteries. A metal Politzer nasal olive was inserted to the patient's nostril and connected to the pump with a Teflon tube. Another Teflon tube was used to connect the pump to a 750 ml GaSampler Single-Patient Collection Bag (Quintron Instrument Company Inc. USA), which is a metalized polyester bag. Small pieces of silicone were then used to connect the tubes to the pump.

To prevent contamination of the air from the pharynx, the soft palate must be closed. Closure of the soft palate can be achieved by the patient blowing against a resistance of at least 10 cm H_2O as instructed by ATS/ERS [17]. In our study, a pressure of 15 cm H_2O was chosen. This was simply performed by measuring 15 centimeters of tap water into a plastic bottle and then asking the patient to blow bubbles in the water. During the aspiration of nasal air, the blowing was supervised by a nurse.

When the pump is started, ambient air is entrained through the patient's open nostril and through the nasal cavity to the contralateral nostril connected with the nasal olive. The seated patient inhales to total lung capacity and then begins to blow against a resistance. At this point, the air in the nasal cavity and the Teflon tubes still contains air from the pharynx. The total length of the Teflon tubes is approximately 500 mm with inner and outer diameters of 6 mm and 8 mm, respectively. Thus, the total volume of the tubes is 14 ml. It has been estimated that the volume of each nasal cavity is approximately 16 ml [18], resulting in a total volume in the nasal cavities and Teflon tubes of approximately 46 ml. The pump can induce a flow of 192 ml/s (11.52 l/min). Therefore, to clear contamination, the suction continues for 1 to 2 seconds while the soft palate is closed before the valve to the bag is opened.

It takes less than 10 seconds to fill the bag. Then, the valve is closed; the patient stops blowing, and the pump is shut down. Each participant used two Teflon tubes which were disposed of after taking the samples.

2.2 DMS device

The DMS device used in this study was the differential ion mobility spectrometer prototype Ionvision (Olfactomics Ltd, Finland). The DMS electrode was 20 mm in length, 8 mm in width, and the analytical gap was 0.25 mm. In DMS, the gas phase molecules are ionized by 4.9 kV soft x-ray. The ions travel in buffer gas in a channel formed by two electrodes, which create an oscillating electric field U_{SV} perpendicular to the motion of the ions. At the end of the channel is a detector, which consists of a Faraday plate connected to a transimpedance amplifier. The electric field has high- and low-voltage phases that cause the ions to travel in a zig-zag motion. If the ions hit the electrodes, they lose their charge before reaching the ion detector. To counter this effect, a compensation voltage U_{CV} is applied. At a certain electric field and compensation voltage value, certain ions reach the detector and generate a pA-range current signal that is detected. Scanning different electric fields and compensation voltages creates a measurement signature that can be presented as a dispersion matrix.

In this study, the samples were scanned with 60 evenly spaced U_{SV} values, ranging from 200 V to 800 V, and 100 evenly spaced U_{CV} values ranging from -1 V to 8 V. Thus, the resultant dispersion field was 800 V/mm – 3.2 kV/mm. The measurement was done simultaneously in positive and negative ion channels, resulting in data vectors of 12 000 dimensions per each measurement in total. The data matrices along with the measurement parameters are then saved as .json files by the DMS device.

2.3 Test participants

We recruited ten adult volunteers to the study. Exclusion criteria were as follows: pregnancy or lactation, smoking during past month, chronic rhinosinusitis, prior

paranasal surgery, acute upper respiratory infection less than a week ago, any use of nasal sprays during the past week, lower respiratory tract disease, such as COPD or asthma, severe immunodeficiency, and any cancer diagnosed within the past five years.

Of the ten participants, four were women and six were men. Mean age was 45 years (range 33 to 64). All participants were able to provide a sample after one attempt. No adverse effects were observed.

2.4 Collection and analysis of the samples

The samples were collected in the same room in the University Hospital to avoid any variation from environmental factors. Each participant provided one nasal air sample on two separate days, resulting in 20 nasal air samples. The collection of samples was completed in five days. Every day, we collected a bag of room air for background VOC comparison, resulting in five room air samples. The bags were connected to the pump with a Teflon tube and a silicone connector in the same manner as the nasal air sampling. However, the pump aspirated room air without having a nasal olive and Teflon tube attached to the inflow port. To remove any VOCs left by the previous participant, the pump was used to aspirate the room air for two minutes between subjects. Each bag was then transported to a separate location and analyzed with the DMS device within six hours, which is the maximum storing time according to the manufacturer of the collection bags.

The collection bag was attached to the DMS device with Teflon tubes. Small pieces of silicone were used between the connections of the tubes. We used a pneumatic ejector VR 05 (Schmalz, Germany) to produce a vacuum for sampling from the bag. Air flow from the sample bag was adjusted to 400 ml per minute with the Gilibrator-2 system (Sensidyne, FL, USA). Pressurized air was also used, and it was cleaned with activated carbon and 5Å molecular sieves. It diluted the sample air to a ratio of 10:1. Thus, total volumetric flow was 4.4 liters per minute. The DMS device can handle an

air flow of 3 liters per minute, so approximately 1.4 liters per minute were lost. Each measurement lasted about 30 seconds, and each collection bag was measured three times while connected to the device. Therefore, the analysis of one bag lasted approximately 1.5 minutes. However, as the volume of the bags were 750 ml and the flow rate was 400 ml per minute, the analysis cycle would require a volume of 1.2 liters. The flow from the sample was not, however, a constant 400 ml per minute because of the potential resistance in the bags when the volume of air was diminishing.

Between measurements of the bags, we measured the room air aspirated through the DMS device. The measurement protocol is shown in figure 1. Of all the measurements, there were 60 nasal air sample measurements and 43 measurements of room air aspirated through the DMS device (termed: reference air). We also had 15 measurements of five bags of room air (termed: room air). However, one measurement was accidentally deleted from the device history, leaving 14 measurements. The day-wise numbers of measurements are presented in table 1.

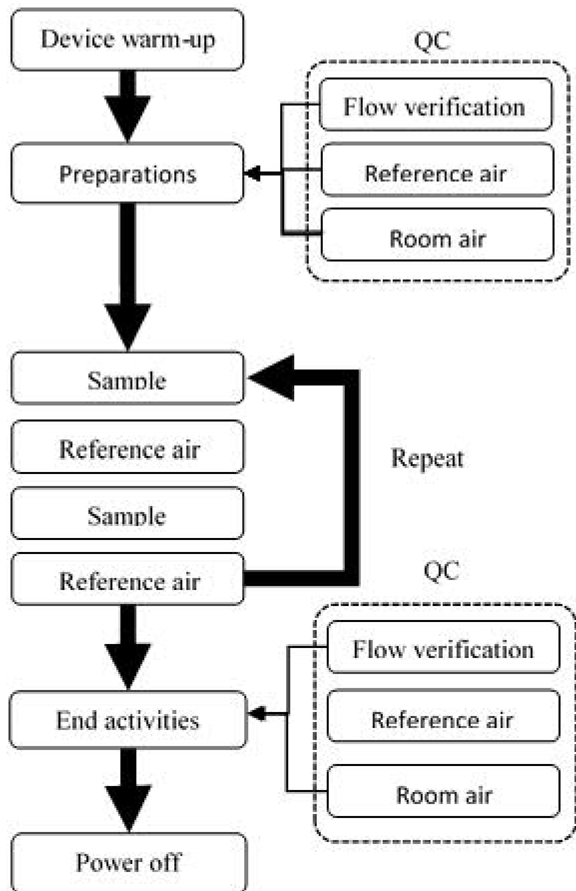

Figure 1. The measurement protocol of the air samples with the differential mobility spectrometry device

Table 1. Number of each measurement type per day.

	Measurement day					
Sample type	Day 1	Day 2	Day 3	Day 4	Day 5	total *n* per sample type
Nasal air	15	9	12	12	12	60
Room air	3	2	3	3	3	14
Reference air	9	7	8	8	11	43
Total *n* per day	27	18	23	23	26	

2.5 Data analysis

The data analysis was performed with a statistical software R [19] in RStudio environment [20]. Packages caret [21], sda [22] and lsa [23] were utilized.

2.6 Data pre-processing

The DMS data were pre-processed by row-wise normalization to emphasize the signals in the high-separation areas on the spectra (figure 2). Each row, corresponding to a fixed U_{SV} value, was scaled between 0 and 1 using the minimum and maximum value of the row. To avoid accidentally emphasizing background noise in the low intensity rows, all values below the pre-defined noise threshold were substituted with the global minimum of the spectra prior to the row-wise normalization. The noise threshold was defined by plotting the histogram of all the intensity data. When the histogram is visually inspected, a gaussian-shaped peak can be observed at the smallest end of the histogram. This is considered to be normally distributed background noise. The values below this visually chosen threshold were substituted with the threshold value.

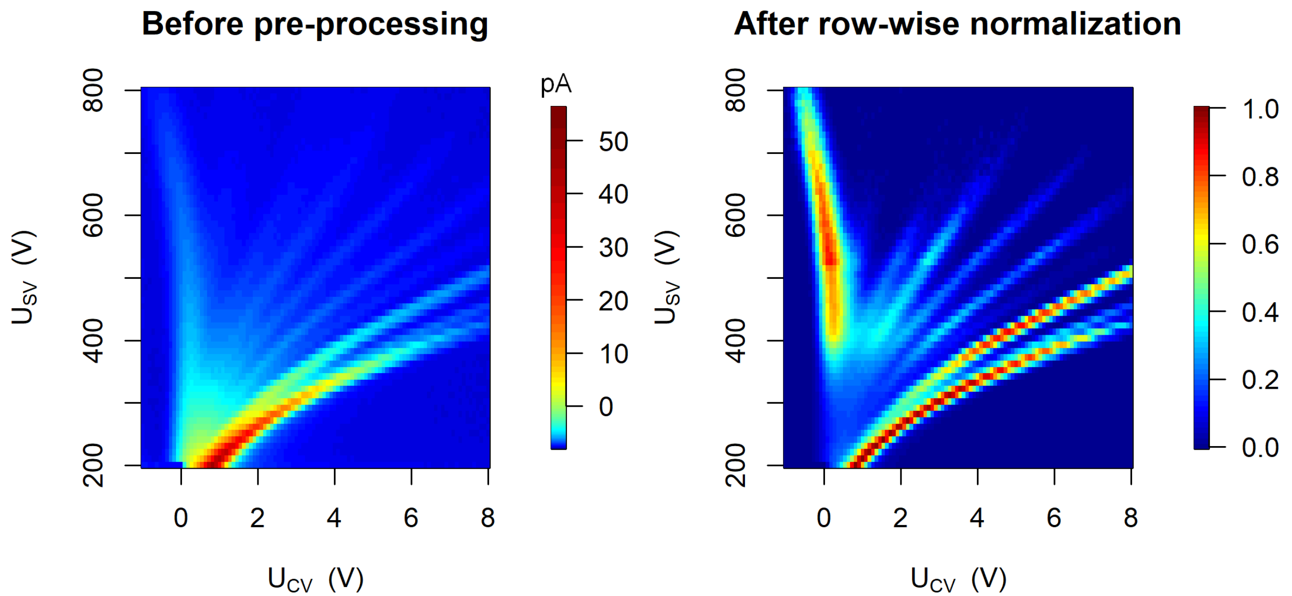

Figure 2. Averaged nasal measurements (negative side) before and after pre-processing and normalization. In the row-normalization, “fragment-like” peaks on the high U_{SV} values are the result of the normalization technique.
pA: picoamperes

2.7 Repeatability verification

To make reliable inference from the DMS measurements, the measurements must be repeatable. Thus, we need a method to compare the similarities and differences between the measurements. In the case of high-dimensional multivariate data (dimensionality d = 12 000), comparison of the measurements is not simple, and traditional univariate testing approaches cannot be used. Therefore, to estimate the repeatability and inter-class similarity of the DMS measurements, several difference and similarity measures were used.

The resemblance of the measurements can be measured with distance metrics, similarity metrics or dissimilarity measures. The distance between two identical measurements is 0, and this distance increases as the measurements are further away

from each other in their feature space. The distance metric used in this study was Euclidean distance, which is the distance between two p-dimensional data vectors x and y, is defined as follows:

$$d(x, y) = \sqrt{\sum_{i=1}^{p}(x_i - y_i)^2} \quad (1)$$

In theory, the upper limit for the distance does not exist. In contrast, similarity between two observations is 1 for identical observations and 0 for completely different observations. To have a comparable "similarity" metric for distance, a concept of dissimilarity (1-similarity) can be used. In this study, dissimilarity versions of cosine similarity as well as Pearson's and Spearman's rank correlation were used.

Our approach was to form an "archetype" for each sample type (nasal air, reference air, room air). In practice, an averaged dispersion plot of each data type was used for this (figure 3). In future studies, this kind of archetype could be used to calibrate the measurement device and the setup. The distance or similarity metric between the new measurements and the archetype can thus be calculated to see whether the new measurements are within the accepted distance/similarity interval.

The effectiveness of this approach was tested by comparing the within-group distances and dissimilarities to the between-group dissimilarities. The nasal air sample data were used to form the archetypes. To avoid bias, a separate archetype was calculated for the nasal air sample data of each measurement day, and the distance was then calculated between the archetypes and each individual measurement from the other days. The distributions of the within-group and the between-group distances and dissimilarities were then compared.

The statistical significance of the findings was tested with Kolmogorov-Smirnov test, which is a general non-parametric statistical test without any distribution assumptions.

2.8 Principal component analysis

Principal component analysis (PCA) is a dimensionality reduction method, where the data are linearly transformed into a feature space that maximizes the variance observed in the data [24]. The first two principal components of the dataset are visualized to illustrate how the data are naturally clustered.

2.9 Classification

Different classification approaches were utilized to find out whether the different measurement clusters were distinguishable from each other. A commonly used way to estimate a classifier's generalization ability to unseen data is cross-validation (CV). In CV, the dataset is divided into k mutually exclusive subsets, and each subset is left out as an independent test set. The rest of the subsets are used to form the model. The overall performance can then be estimated from the combined test results of the subsets. If the measurements are independent, the subsets (folds) can be formed by random split (k-fold CV), or each instance can even form a subset of its own (in which case it is called leave-one-out CV, LOOCV). In our case, however, the measurements are not independent: the measurement order, the measurement day and the participant all compromise the independence. Thus, CV was performed by dividing the data into day-wise or participant-wise folds.

Linear discriminant analysis (LDA) is a classification method, where the classes are separated by hyperplanes maximizing the class separation. Due to the high dimensionality of the data ($d = 12\,000$), regularization is required. The regularized version of LDA is shrinkage LDA (sLDA), which has previously been applied successfully to classify DMS data [25, 26], and was used in this study, too.

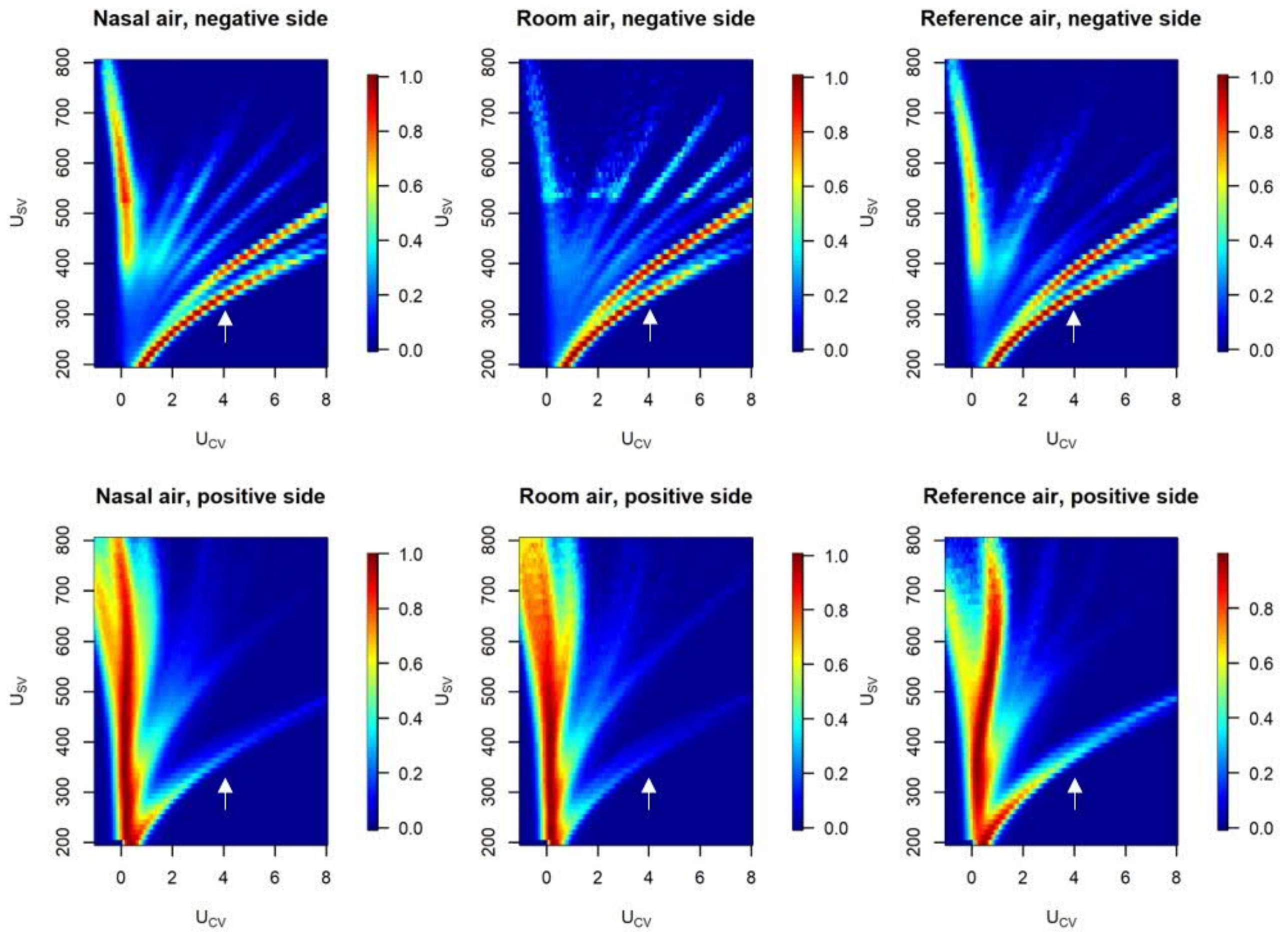

Figure 3. The averaged dispersion plots of each sample type after pre-processing with row-normalization technique. Reactant ion peak (a water peak) that resembles water in the spectrum is shown with a white arrow.

3. Results

PCA decomposition creates insight into the inherent clustering of the data (figure 4). The plots show that the nasal air measurements are distinguishable from the reference and room air measurements, while the measurement day also affects the measurements (figure 4a and 4b). In the nasal air data (figure 4c), it seems that even though the three nasal air measurements from the same bag are usually observed close together, there is no participant-wise clustering if the measurements of both bags of the same participant are studied.

The leave-one-day-out cross-validation results of the sLDA classifier for the sample types are shown in table 2. Each model was able to correctly classify 100% of the out-of-sample data. This means that the data were perfectly linearly separable.

Discrimination between study participants with sLDA was cross-validated by a 2-fold setup, where the first fold consisted of the first sample bags of each participant, and the second fold contained the latter measurement bags. The discrimination rate was 13.3% and, as such, does not significantly differ from the guess level of 10%. Visual assessment on the PCA plot (figure 4c) does not reveal significant clustering by a participant.

The boxplots of the distance and the dissimilarity metrics between the data groups and the nasal air sample archetype are shown in figure 5. The within-group distances and dissimilarities were notably lower than the corresponding between-group metrics in all cases. The differences between the distributions of the archetype class and the other types were statistically significant on a 95% significance level in all cases. Kolmogorov-Smirnov test showed statistically significance ($p < 0.05$) between all sample types.

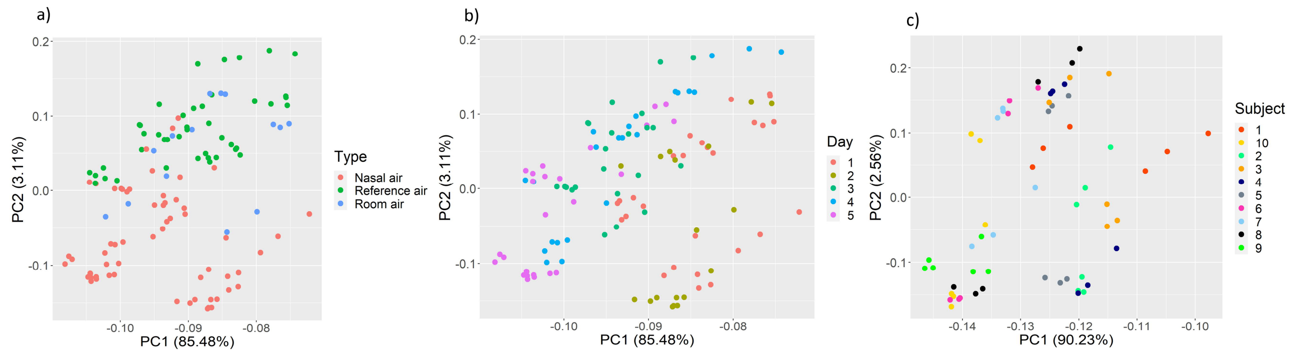

Figure 4. Two first principal components of a) the entire dataset, grouped by the measurement type, b) the entire dataset, grouped by the measurement day and c) the nasal air samples grouped by participants.

		True class		
		Nasal air	Room air	Reference air
Predicted class	Nasal air	60	0	0
	Room air	0	14	0
	Reference air	0	0	43

Table 2. Day-wise cross-validation results of the sample type classification with a shrinkage linear discriminant analysis model.

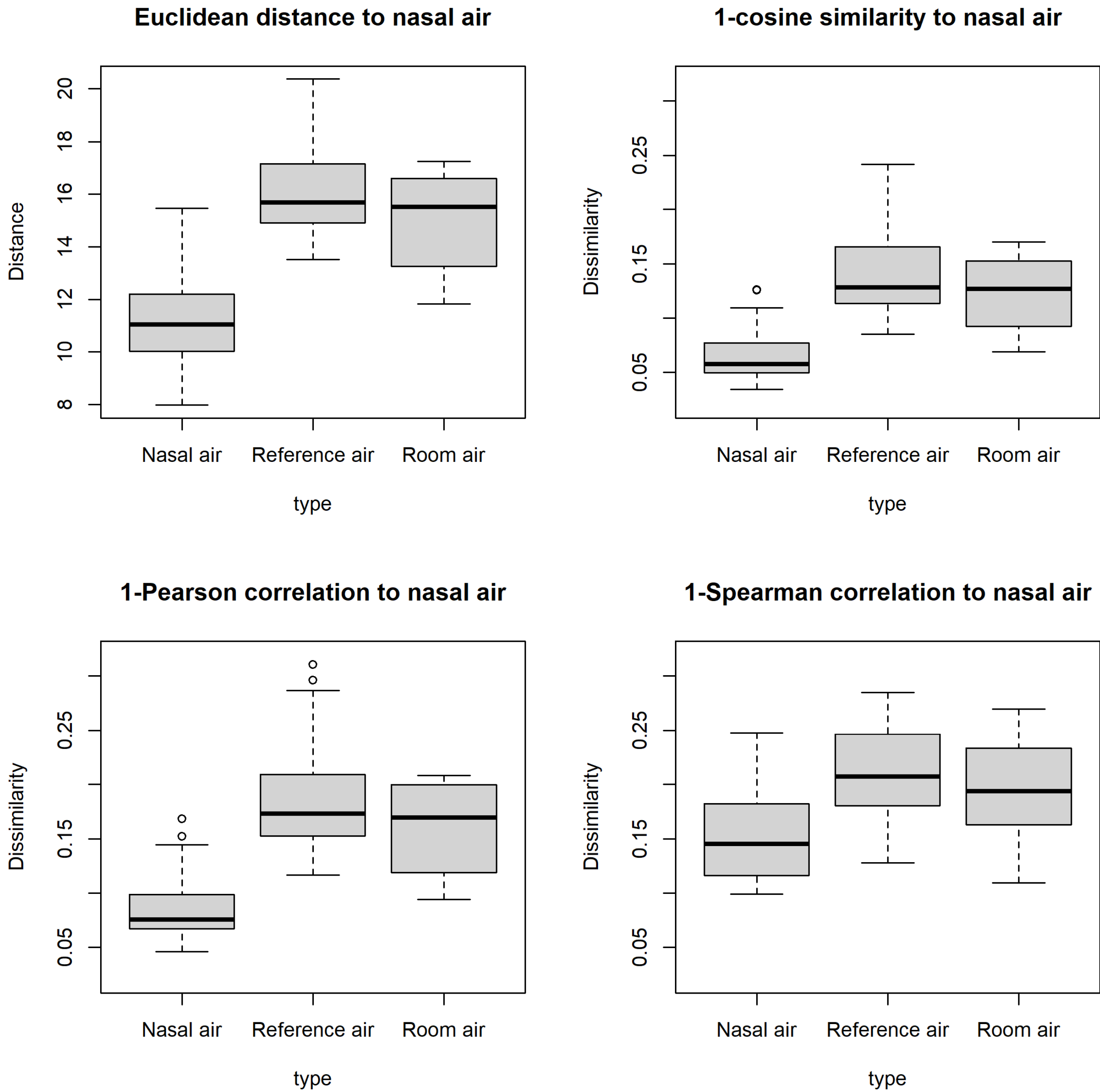

Figure 5. The boxplots of the cross-validated distance and dissimilarity metrics between the data groups and the nasal air sample archetype. The dissimilarity metrics all have the same scale on the vertical axis, whereas the Euclidean distance has its own, non-comparable scale.

The absolute humidity of the diluted sample types is shown in figure 6. Nasal air is more humid than reference and room air but no larger than variance of the humidity between measurement days. This is also illustrated by the visual intensity of a reaction ion peak in figure 3.

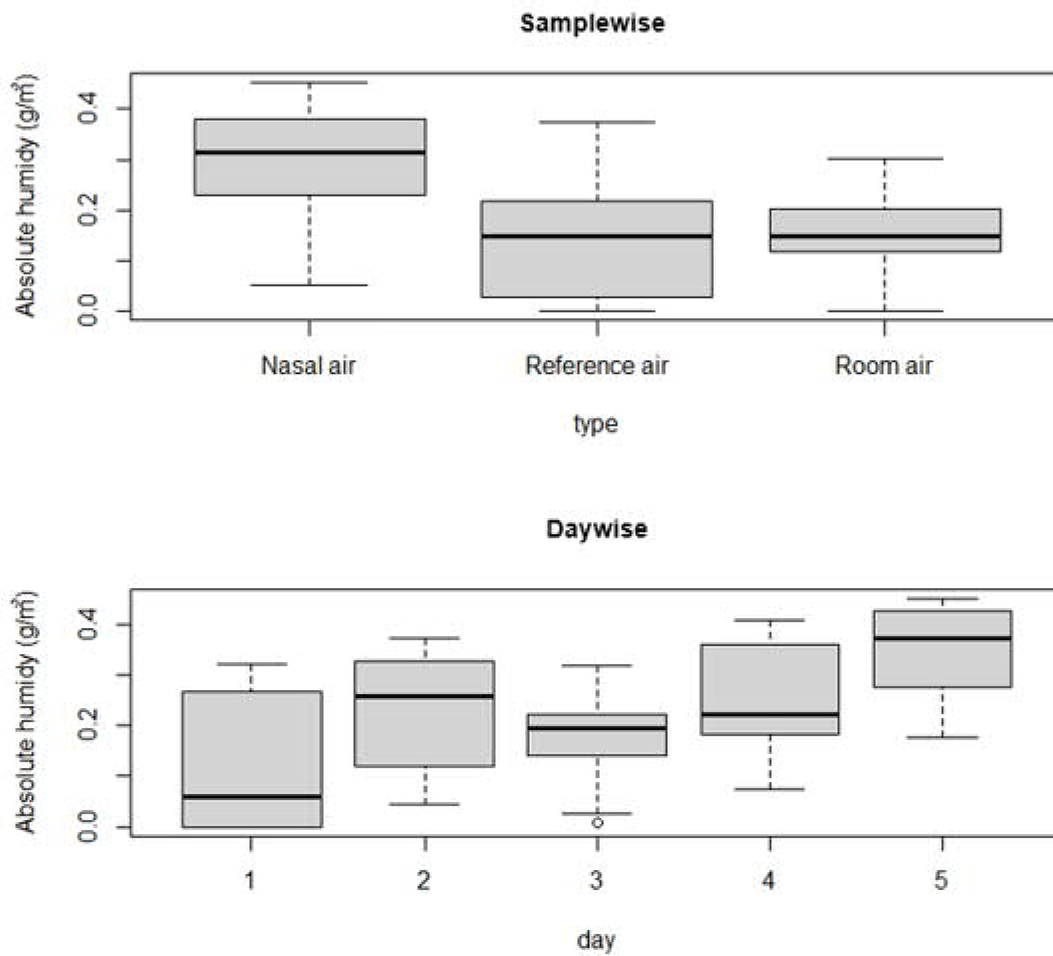

Figure 6. Absolute humidity in diluted air showing variance between samples and measurement days.

4. Discussion

In this study, we evaluated a method to collect and analyze nasal air that resembles the ATS/ERS guidelines for the measurement of nasal NO. Data analysis showed that nasal air, room air and reference air were clearly separable and established reference intervals for measurements from healthy participants. As the participants were not distinguishable from each other, there was no clustering of the data based on individual characteristics but only by sample type.

As we examined only healthy individuals, we do not know whether the analysis of nasal air can distinguish patients with conditions from healthy ones. To our knowledge, only a few studies have examined nasal air analysis in the diagnostics of diseases with eNoses [11-14]. Mohamed *et al.* [11] collected nasal outbreath into sterile plastic sacks from five chronic rhinosinusitis patients and five controls. Patients inhaled through the mouth and exhaled through the nose to fill the sack. In addition, the plastic sack contained a tampon that was first held in the middle meatus of the patient's nose to stimulate mucosal secretions. The contents of the sack were then analyzed using an eNose developed for research purposes (LibraNose, University of Rome Tor Vergata and Technobiochip) that utilizes quartz crystal microbalances covered with metalloporphyrins. The eNose had a sensitivity and specificity of 60% after leave-one-out cross-validation. Thus, the results did not differ much from the guess level of 50%. In the other study, breath samples from patients with acute rhinosinusitis symptoms and controls were obtained using a modified nasal CPAP mask that was connected to an eNose based on conducting polymer sensors. After leave-one-out cross-validation, the eNose could diagnose bacterial rhinosinusitis with an accuracy of 72% [12]. Both the aforementioned studies examined exhaled nasal air coming directly from the lungs, the pharynx and the oral cavity, which may have caused confusing results. However, Steppert *et al.* had more promising results in two recent pilot studies investigating nasal air with an IMS

coupled with a multicapillary column [13, 14]. In their first study, nasal air was aspirated during normal respiration. Samples were collected from individuals with confirmed influenza-A infection and then compared to persons with negative test results and to healthy volunteers. Influenza-A-infected patients were distinguished with perfect sensitivity and specificity [13]. Furthermore, the second study showed that an analysis of exhaled nasal air from patients with SARS-CoV-2 infection could be distinguished from patients with influenza-A infection and healthy controls with accuracy of 97% after cross-validation [14].

We consider it crucial to investigate aspirated nasal air in studies concerning rhinologic diseases to exclude confounding factors as much as possible. Guidelines suggest the application of aspiration in the measurement of nasal NO [17], which is also applicable for nasal air analysis. This method excludes sample contamination with air originating from the lungs, the pharynx or the oral cavity, as it could affect the VOC profile. For instance, Smith *et al.* [27] examined ammonia levels in breath air and found that levels are significantly less in nose-exhaled breath than mouth-exhaled breath. The same applies for ethanol and hydrogen cyanide [28]. Because ion mobility spectrometry is sensitive for these compounds, they could cause significant bias if left unchecked [29-31]. Therefore, an analysis of nasal air could reduce contamination by endogenous VOCs that originate in the oral cavity. In exhaled breath sampling, VOCs depend on which portion of breath is analyzed. Alveolar samples, for example, show different VOCs than mixed expiratory samples [15]. Also, expiratory flow rate and breath hold influence the eNose pattern [16]. Aspiration of the nasal air does not suffer from these problems. However, one should note the potential confounding factors of aspiration. First, we did not measure nasal CO_2, which, when remaining low, would verify the closure of the soft palate. Nevertheless, blowing against resistance of a minimum of 10 cm H_2O is approved to be adequate [17]. Second, the flow of the air was not measured, and it could be affected by nasal aerodynamics. As the batteries of the pump were running out, a

reduction in the flow of the air was observed. Measurements of nasal NO suggest a targeted airflow, otherwise the values of NO are affected [17]. Similarly, the signal patterns of the eNose could be altered.

Many patient-related factors can affect the breath analysis regardless of whether the sample is collected through the mouth or the nose. For example, the consumption of certain foods can affect VOCs [32]. Some studies even advocate fasting before sample collection [7, 33, 34], but the role of fasting or diet on VOCs is unclear [35]. Moreover, we are unaware of any previous studies that have compared the effects of diet on the nasal and oral sampling of air. Other possible covariates that alter VOCs include age, gender, smoking status, and comorbid diseases. However, controversy exists as to which of these covariates should be adjusted for breath analysis [4, 34, 36-39]. Also, medication, such as nasal sprays, could affect the VOC profile. Indeed, nasal decongestants and corticosteroid sprays have been shown to decrease nasal NO [40-42]. In our study, we advised the volunteers to refrain from using their nasal sprays for a week prior to sample collection, but a shorter period would probably have been sufficient. However, corticosteroid sprays have a prolonged effect on inflammation, and the time the sprays take to wear-off is unknown.

Room air is a source of exogenous VOCs that might interfere with the results. A typical way to exclude the impact of room air is to use an inspiratory VOC filter and to rinse the patient's lungs with filtered air [43]. In our study, this would have demanded the use of a filter attached to the patient's open nostril. To our knowledge, it is not known how much time would be enough to rinse the nasal cavity with purified air. As we used an estimate of 32 ml for the volume of both nasal cavities [18], we expect that a few seconds of aspiration would replace the room air in the nasal cavity with purified air. Nevertheless, one should note that use of a clean air supply might be an additional confounding factor since it might reduce concentrations of likely endogenous VOCs and increase exogenous [44]. Furthermore, breath collection devices can release contaminant VOCs [45], which

also applies to our pump. Therefore, if one would want to eliminate its effect, an airtight container should be used. The container has the sampling bag inside and two airtight ports. One port connects to a pump outside the container and the other to a bag to supply sample air from the patient via a tube. When the pump is turned on, the air in the container is drawn out, which produces a differential pressure, and air is then drawn into the bag via the port from the patient's nose. This method would not, however, allow cleaning of the nasal cavity from air of the oral cavity while the patient is blowing against resistance at the beginning of nasal air aspiration. In addition to the pump, the tubes are also a potential source of contamination. In the present study, we used Teflon tubes which were disposed of after the test. Teflon is a suitable material due to it being inert and is suitable for use with the eNose [46]. Teflon is, however, quite rigid and requires more adjustment with the pump compared to silicone.

It should be borne in mind that in the present study air samples were stored in the collection bags. Previous studies have shown that VOCs adsorb from bags over time, which affects the storing time [47, 48]. Therefore, we analyzed all samples as quickly as possible and within at least 6 hours, as instructed by the manufacturer of the bags. Furthermore, we did not re-use the collection bags, although with cleaning protocols it would have been possible and would have reduced costs [48, 49]. However, cleaning may still fail to remove some compounds [50]. During the analysis of the bags with the DMS device, the flow from the sample was set to 400 ml per minute, but the resistance in the bags during emptying varied and affected the flow rate. Therefore, clean air was most likely present in different volumes in the three measurements of the one bag. This did not, however, seem to have a significant effect since the PCA composition shows that the measurements are usually close to each other.

Since some environmental factors, such as temperature, humidity and air quality, cannot always be controlled in a clinical setup, they can be expected to affect the

DMS measurements in some way. The DMS is sensitive to humidity and therefore measurements of the same compound in different humidity levels might produce different results. However, the air in the nasal cavity is saturated to between 90% and 100% [51, 52]. Therefore, changes in the humidity of room air supposedly does not significantly affect the measurements of nasal air. The measurement device itself can also produce dynamically changing baseline noise to the measurements due to system stabilization. The changes in the baseline can be compensated by using various normalization methods. The row-wise normalization used in this study highlights the higher parts of the DMS dispersion matrices, where the peak separation is the highest but the signal is the weakest. With this method, the sample types became perfectly linearly separable.

A potential reason for the separability of nasal air from the reference and room air is that nasal air contains endogenous VOCs and the concentration of some VOCs of the room air might change during the air flow through the nasal cavity. Most importantly, the air is humidified in the nasal cavity during aspiration to the collection bag. As seen from the figure 6, nasal air is more humid than other samples but the humidity also varies greatly between measurement days. Although the reaction ion peak that resembles water in the spectrum differs between different sample types, there is significant variation caused by other compounds in other areas of the spectrum as well (figure 3). Because the measurement device was located in a different location compared to the collection of the nasal air and room air samples (hospital environment), the VOCs in the reference air were different, which may explain the differences in the box plots. Although we recognize that the strength of our study is limited by the small sample size, the study still manages to achieve good, unbiased results despite this limitation.

The data analysis also had possible bias factors. The most obvious bias factor results from the nasal air sample bags, each of which were measured three times on the same

day. They are expected to be highly similar to each other, and this is also supported by the PCA transformation of the nasal air data, where the measurements from the same bag are usually observed close together. Furthermore, the measurement order of the different samples (figure 1) was always the same and could therefore cause bias and affect the distances and classification results. Another factor is the measurement day. The measurement conditions during a measurement session are similar between measurements, which is why measurements from the same session tend to cluster together. Thus, to avoid bag-wise and day-wise bias, the cross-validation was performed by leaving each day as a test set at a time. However, since the nasal air of each participant was measured twice on separate days, each test day contained data from the same participants that were also present in the training data. Even though the participant-wise measurements did not form participant-wise clusters in the visual inspection of the PCA, this is still a possible bias factor in the sLDA classification. However, since the measurement types did not differ significantly between days, this is unlikely to be a great disadvantage.

The distance comparisons show that the distance and dissimilarity metrics, especially Euclidean distance where the relative differences between the archetype group and the other groups are most prominent, could be used to study the repeatability of the DMS data and in device calibration. The absolute values of the Euclidean distances cannot be directly compared to the dissimilarities since the scales differ. However, as Euclidean distance is widely used in different fields and it is intuitively simple to understand, we recommend its use. Moreover, all the presented metrics are computationally cheap to evaluate.

Breath analysis for disease detection is an exciting and promising field of research. Although it is important to find disease-specific biomarkers using, e.g., mass spectrometry, the qualitative analysis of breath based on pattern recognition better suits fast and cheap point-of-care use. As there is a lack of standardization in breath

sampling, we evaluated a method that is similar to the validated method for the measurement of nasal NO to diminish confounding factors. We believe that this kind of approach is suitable for use in the diagnostics of rhinologic diseases, such as acute and chronic rhinosinusitis, allergic rhinitis and sinonasal cancers.

5. Conclusion

Although numerous studies on exhaled breath analysis exist, this is the first study to examine the eNose analysis of aspirated nasal air with soft velum closed. The study shows that the concept of collecting nasal air into a breath collecting bag by aspiration and the subsequent analysis of the nasal air using DMS works well. Indeed, DMS distinguishes sample types perfectly but the difference in the humidity of the samples might contribute to the results. We believe that the analysis of aspirated nasal air with DMS brings more potential for the use of the method in disease detection studies.

Acknowledgements

Conflicts of interest

Markus Karjalainen, Anton Kontunen, Niku Oksala and Antti Roine are shareholders in Olfactomics Ltd, which is about to commercialize proprietary technology for the detection of diseases using ion mobility spectrometry. Anna Anttalainen is an employee in Olfactomics Ltd. The remaining authors have no conflict of interests to declare.

Funding

Jussi Virtanen declares funding from the Doctoral School of Tampere University, the Finnish Medical Foundation, the Finnish ORL-HNS Foundation and Tampere Tuberculosis Foundation. This study was financially partly supported by the Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospital. These organizations had no role in the design or execution of the study or in the writing process of the manuscript.

Ethical statement

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was conducted according to medical device trial regulations and was approved by the National Supervisory Authority for Welfare and Health. All volunteers provided written informed consent.

References

1. Sethi S, Nanda R, Chakraborty T 2013 Clinical application of volatile organic compound analysis for detecting infectious diseases *Clin. Microbiol. Rev*. **26** 462–75

2. Wilson AD 2015 Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath *Metabolites* **5** 140–63

3. Phillips M, Herrera J, Krishnan S, Zain M, Greenberg J, Cataneo RN 1999 Variation in volatile organic compounds in the breath of normal humans *J. Chromatogr. B Biomed Sci. Appl*. **729** 75–88

4. Chen X, Wang F, Lin L, Dong H, Huang F, Muhammad KG, Chen L, Gorlova OY 2017 Association of smoking with metabolic volatile organic compounds in exhaled breath *Int. J. Mol. Sci.* **18** 2235

5. Park SY, Kim Y, Kim T, Eom TH, Kim SY, Jang HW 2019 Chemoresistive materials for electronic nose: progress, perspectives, and challenges *InfoMat* **1** 289–316

6. Dodds JN, Baker ES 2019 Ion mobility spectrometry: fundamental concepts, instrumentation, applications, and the road ahead *J Am Soc Mass Spectrom*. **30** 2185–2195

7. Fens N, Roldaan AC, van der Schee MP, Boksem RJ, Zwinderman AH, Bel EH, Sterk PJ 2011 External validation of exhaled breath profiling using an electronic nose in the discrimination of asthma with fixed airways obstruction and chronic obstructive pulmonary disease *Clin. Exp. Allergy* **41** 1371–8.

8. Roine A *et al*. 2014 Detection of prostate cancer by an electronic nose: a proof of principle study *J. Urol.* **192** 230–5

9. Niemi RJ, Roine AN, Eräviita E, Kumpulainen PS, Mäenpää JU, Oksala N 2018 FAIMS analysis of urine gaseous headspace is capable of differentiating ovarian cancer *Gynecol Oncol.* **151** 519–524

10. Farraia MV, Cavaleiro Rufo J, Paciência I, Mendes F, Delgado L, Moreira A 2019 The electronic nose technology in clinical diagnosis *Porto Biomed J.* **4** e42

11. Mohamed EI, Bruno E, Linder R, Alessandrini M, Di Girolamo A, Pöppl SJ, Puija A, De Lorenzo A 2003 A novel method for diagnosing chronic rhinosinusitis based on an electronic nose *An. Otorrinolaringol. Ibero Am.* **30** 447–57

12. Thaler ER, Hanson CW 2006 Use of an electronic nose to diagnose bacterial sinusitis *Am. J. Rhinol.* **20** 170–2

13. Steppert C, Steppert I, Bollinger T, Sterlacci W 2020 Rapid non-invasive detection of Influenza-A-infection by multicapillary column coupled ion mobility spectrometry *J Breath Res.* **22** 011001

14. Steppert C, Steppert I, Sterlacci W, Bollinger T 2021 Rapid detection of SARS-CoV-2 infection by multicapillary column coupled ion mobility spectrometry (MCC-IMS) of breath. A proof of concept study *J Breath Res.* **15** 027105

15. Miekisch W, Kischkel S, Sawacki A, Liebau T, Mieth M, Schubert JK 2008 Impact of sampling procedures on the results of breath analysis *J. Breath Res.* **2** 026007

16. Bikov A, Hernadi M, Korosi BZ, Kunos L, Zsamboki, G, Sutto Z, Tarnoki AD, Tarnoki DL, Losonczy G, Horvath I 2014 Expiratory flow rate, breath hold and anatomic dead space influence electronic nose ability to detect lung cancer *BMC Pulmonary Medicine* **14** 202

17. American Thoracic Society, European Respiratory Society 2005 ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide *Am. J. Respir. Crit. Care Med.* **171** 912–30

18. Valtonen O, Ormiskangas J, Kivekäs I, Rantanen V, Dean M, Poe D, Järnstedt J, Lekkala J, Saarenrinne P, Rautiainen M 2020 Three-dimensional printing of the nasal cavities for clinical experiments *Sci Rep.* **16** 502

19. R Core Team 2020 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

20. RStudio Team 2020 RStudio: Integrated Development for R. RStudio, PBC, Boston, MA http://www.rstudio.com/.

21. Kuhn M 2020 caret: Classification and Regression Training. R package version 6.0-86. https://CRAN.R-project.org/package=caret

22. Ahdesmäki M, Zuber V, Gibb S, Strimmer K 2015 sda: Shrinkage Discriminant Analysis and CAT Score Variable Selection. R package version 1.3.7. https://CRAN.R-project.org/package=sda

23. Wild F 2020 lsa: Latent Semantic Analysis. R package version 0.73.2. https://CRAN.R-project.org/package=lsa

24. Pearson K 1901 LIII. On lines and planes of closest fit to systems of points in space *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **2** 559–72

25. Kontunen A, Karjalainen M, Lekkala J, Roine A, Oksala N 2018 Tissue identification in a porcine model by differential ion mobility spectrometry analysis of surgical smoke *Ann. Biomed. Eng.* **46** 1091–1100

26. Sutinen M, Kontunen A, Karjalainen M, Kiiski J, Hannus J, Tolonen T, Roine A, Oksala N 2019 Identification of breast tumors from diathermy smoke by differential ion mobility spectrometry *Eur. J. Surg. Oncol.* **45** 141–146

27. Smith D, Wang T, Pysanenko A, Spanel P 2008 A selected ion flow tube mass spectrometry study of ammonia in mouth- and nose-exhaled breath and in the oral cavity *Rapid Commun. Mass Spectrom.* **22** 783–9

28. Wang T, Pysanenko A, Dryahina K, Spaněl P, Smith D 2008 Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity *J. Breath Res.* **2** 037013

29. Myles L, Meyers, T, Robinson L 2006 Atmospheric ammonia measurement with an ion mobility spectrometer *Atmospheric Environment* **40** 5745–52

30. Sielemann St, Baumbach JI, Schmidt H, Pilzecker P 2001 Detection of alcohols using UV-ion mobility spectrometers *Analytica Chimica Acta* **431** 293–301

31. Seto Y *et al*. 2019 Development of ion mobility spectrometry with novel atmospheric electron emission ionization for field detection of gaseous and blister chemical warfare agents *Anal. Chem*. **91** 5403–14

32. Krilaviciute A *et al.* 2019 Associations of diet and lifestyle factors with common volatile organic compounds in exhaled breath of average-risk individuals *J. Breath Res.* **13** 026006

33. Gruber M *et al.* 2014 Analysis of exhaled breath for diagnosing head and neck squamous cell carcinoma: A feasibility study *Br. J.Cancer* **111** 790–8

34. Dragonieri S, Quaranta VN, Carratu P, Ranieri T, Resta O 2016 Influence of age and gender on the profile of exhaled volatile organic compounds analyzed by an electronic nose *J. Bras. Pneumol.* **42** 143–5

35. Jia Z, Patra A, Kutty VK, Venkatesan T 2019 Critical review of volatile organic compound analysis in breath and in vitro cell culture for detection of lung cancer *Metabolites* **9** 52

36. Kischkel S, Miekisch W, Sawacki A, Straker EM, Trefz P, Amann A, Schubert JK 2010 Breath biomarkers for lung cancer detection and assessment of smoking related effects - confounding variables, influence of normalization and statistical algorithms *Clin. Chim. Acta* **411** 1637–44

37. Filipiak W, Ruzsanyi V, Mochalski P, Filipiak A, Bajtarevic A, Ager C, et al. 2012 Dependence of exhaled breath composition on exogenous factors, smoking habits and exposure to air pollutants *J. Breath Res.* **6** 036008

38. Broza YY, Braverman I, Haick H 2018 Breath volatolomics for diagnosing chronic rhinosinusitis *Int. J. Nanomedicine* **13** 4661–70

39. Tiele A, Wicaksono A, Kansara J, Arasaradnam RP, Covington JA 2019 Breath analysis using enose and ion mobility technology to diagnose inflammatory bowel disease — A pilot study *Biosensors* **9** 1–16

40. Dillon WC, Hampl V, Shultz PJ, Rubins JB, Archer SL 1996 Origins of breath nitric oxide in humans *Chest* **110** 930–8

41. Chatkin JM, Djupesland PG, Qian W, McClean P, Furlott H, Gutierrez C, Zamel N, Haight JS 1999 Nasal nitric oxide is independent of nasal cavity volume *Am. J. Rhinol.* **13** 179–84

42. Vural C, Gungor A 2003 The effect of topical fluticasone on nasal nitric oxide levels in a patient with allergic rhinitis *Ear Nose Throat J.* **82** 592–7

43. Dragonieri S *et al.* 2007 An electronic nose in the discrimination of patients with asthma and controls *J. Allergy Clin. Immunol.* **120** 856–62

44. Di Gilio A *et al.* 2020 Breath analysis: comparison among methodological approaches for breath sampling *Molecules* **10** 5823

45. Doran SLF, Romano A, Hanna GB 2017 Optimisation of sampling parameters for standardized exhaled breath sampling *J. Breath Res.* **6** 016007.

46. Karjalainen M, Kontunen A, Mäkelä M, Anttalainen O, Vehkaoja A, Oksala N, Roine A 2020 Recovery characteristics of different tube materials in relation to combustion products *Int. J. Ion Mobil. Spec.* **23** 83–90

47. Pet'ka J, Étievant P, Callement G 2000 Suitability of different plastic materials for head or nose spaces short term storage *Analusis* **28** 330-35

48. Mochalski P, Wzorek B, Śliwka I, Amann A 2009 Suitability of different polymer bags for storage of volatile sulphur compounds relevant to breath analysis *J. Chromatogr. B. Analyt. Technol. Biomed Life Sci.* **877** 189–96

49. Beauchamp J, Herbig J, Gutmann R, Hansel A 2008 On the use of Tedlar® bags for breath-gas sampling and analysis *J Breath Res.* **2** 046001

50. McGarvey LJ, Shorten CV 2000 The effects of adsorption on the reusability of Tedlar® air sampling bags *AIHAJ - American Industrial Hygiene Association* **61** 375-380

51. Rouadi P, Baroody FM, Abbott D, Naureckas E, Solway J, Naclerio RM 1999 A technique to measure the ability of the human nose to warm and humidify air *J. Appl. Physiol. (1985)* **87** 400-6

52. Lindemann J, Leiacker R, Rettinger G, Keck T 2003 The relationship between water vapour saturation of inhaled air and nasal patency *Eur Respir J.* **21** 313-6