



Electrostatic Sampling of Patient Breath for Pathogen Detection: A Pilot Study

Laila Ladhani¹, Gaspard Pardon¹, Pieter Moons², Herman Goossens² and Wouter van der Wijngaart^{1*}

¹ Micro and Nanosystems, KTH Royal Institute of Technology, Stockholm, Sweden, ² Department of Medical Microbiology, University of Antwerp, Antwerp, Belgium

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*Correspondence:

Wouter van der Wijngaart
wouter@kth.se

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Collecting samples for diagnosis of respiratory infections can be invasive and highly uncomfortable for patients; with sampling techniques ranging from nasal or throat swabs to bronchoalveolar lavage. In this work, we explore the electrostatic capture of exhaled pathogens as a non-invasive sampling method for rapid diagnosis in a primary care setting. A pilot study at primary care centers in Belgium enrolled 20 patients presenting with flu-like symptoms whose diagnosis was determined by nasopharyngeal (NP) swabs. We collected exhaled aerosol particles from infected patients using a breath electrostatic sampler (BESS) and using filters, following an extremely light procedure consisting of five normal exhales. All samples were analyzed using a commercial multiplex q-RT-PCR panel assay. *Staphylococcus aureus* (*S. aureus*) was detected from BESS samples of seven patients; three of whom were in agreement with their corresponding diagnoses. The BESS method was negative for exhaled viruses. We detected viruses, but no bacteria, with the filters, but the results showed no correlation with the corresponding diagnosis. Our results indicate that electrostatic sampling of exhaled breath is a technique and approach potentially suited for the primary care setting, where it might constitute a helpful diagnostic device.

Keywords: exhaled breath aerosol, electrostatic sampling, point-of-care test, non-invasive diagnostics, respiratory infectious diseases

INTRODUCTION

Exhaled breath analysis is a developing field with the possibility to provide individualized and non-invasive disease diagnostics. Traditionally, diagnosis of exhaled breath only considered the analysis of volatile organic compounds (VOCs) and gases. During the last 25 years, breath research has expanded beyond gas-phase analysis to include expelled aerosols containing non-volatile compounds, such as lipids, proteins, DNA and bacteria (Pleil et al., 2018). Much effort has been put into analyzing these non-volatile biomarkers, mostly using condensation capture methods (Davis et al., 2012), but often relying on detecting a set of various volatile biomarkers to determine the disease state of a patient. With the emergence of cheap, highly sensitive and miniaturized molecular diagnostic technologies, an interesting alternative is the capture and direct detection of specific disease-causing pathogens in exhaled aerosol (Brankston et al., 2007; Fabian et al., 2011; Johnson et al., 2011). Such tests could prove extremely helpful in the case of epidemics, such as previous Influenza (Yan et al., 2018), Severe Acute Respiratory Syndromes (SARS) (Booth et al., 2005; Chowell et al., 2015) or Corona virus outbreaks (Santarpia et al., 2020).

To capture aerosolized pathogens from exhaled breath, several techniques have emerged (Grinshpun et al., 1996; Fung and Mykhaylova, 2013): impactors (Fennelly et al., 2004; Wainwright et al., 2009; Gralton et al., 2013), filters (Fabian et al., 2008; McDevitt et al., 2013; Hatagishi et al., 2014), liquid impingers (Lindsley et al., 2010) and condensators (Xu et al., 2012; Zheng et al., 2018). An alternative method for sampling aerosols has yet to be considered more thoroughly: electrostatic sampling (Parker, 2003). Electrostatic sampling has the advantages of handling high-volume air flows, compatible with human exhaled stream, and has demonstrated high capture efficiencies (>90% for particles larger than $1\ \mu\text{m}$; Parker, 2003). Compared to impaction devices, electrostatic samplers have a lower impaction velocity (Mainelis, 1999; Mainelis et al., 2002) and pressure drop, which could play a role in preserving the integrity of pathogens. Furthermore, compared to liquid impingers, electrostatic samplers have demonstrated 5–10 times higher concentration output (Yao et al., 2009; Wei et al., 2014; Rufino de Sousa et al., 2020). There is potential to develop this technology for clinical use as an alternative sampling technique for exhaled breath aerosols (Han and Mainelis, 2008; Kettleson et al., 2009; Miller et al., 2010; Christensen et al., 2011; Tan et al., 2011; Lancereau et al., 2013); however, we found no reports yet of direct exhaled breath sampling using electrostatic methods.

In this work, we performed a clinical pilot study in which we electrostatically captured breath aerosols from infected patients at a point-of-care setting (Figure 1). Additionally, we aimed to validate the electrostatic sampling device for the capture

of pathogens from exhaled breath. We adapted a previously developed electrostatic sampler for *in-vitro* capture of aerosolized influenza virus (Pardon et al., 2015; Ladhani et al., 2017) into a Breath ElectroStatic Sampler (BESS) for patient use. We report, to our knowledge, the first investigation of breath aerosol sampling using an electrostatic sampler.

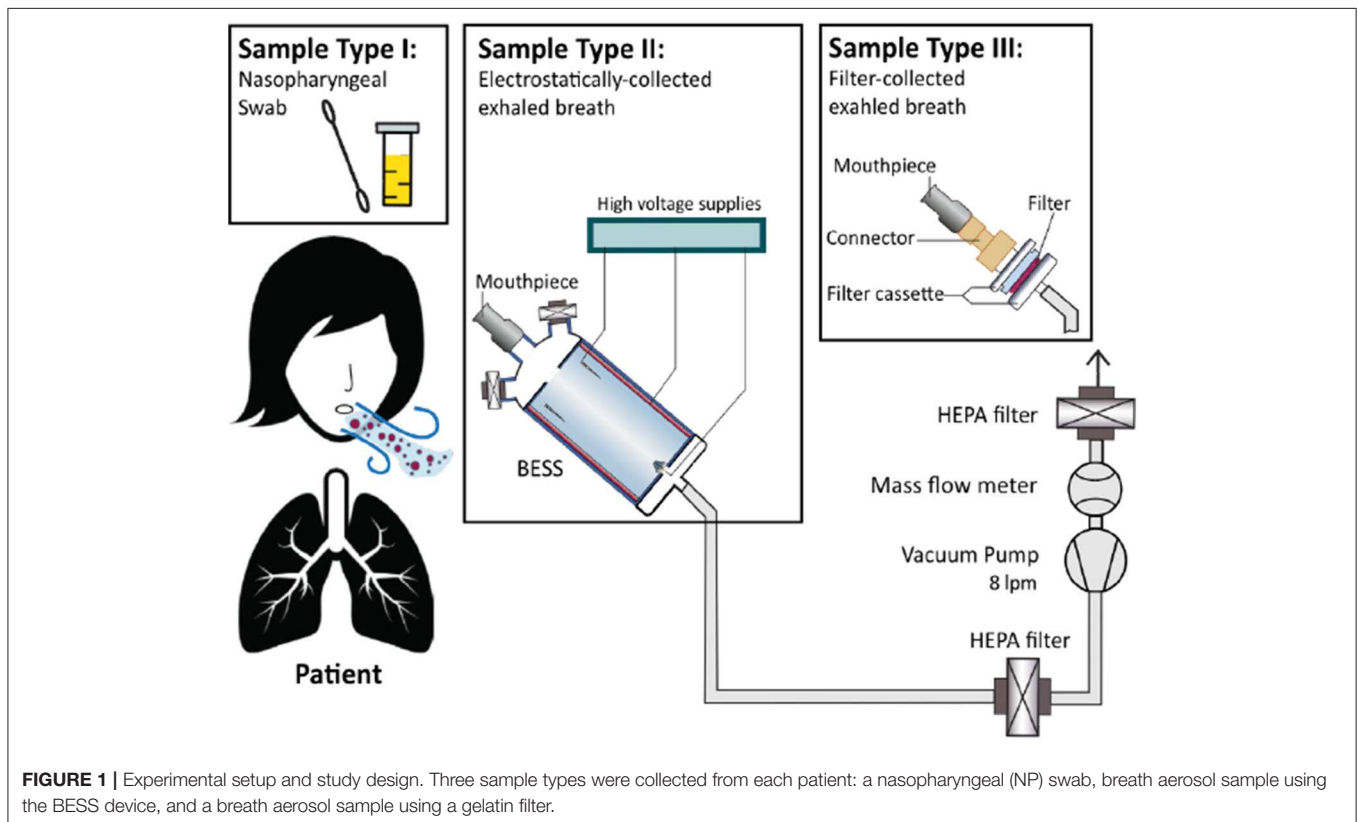
MATERIALS AND METHODS

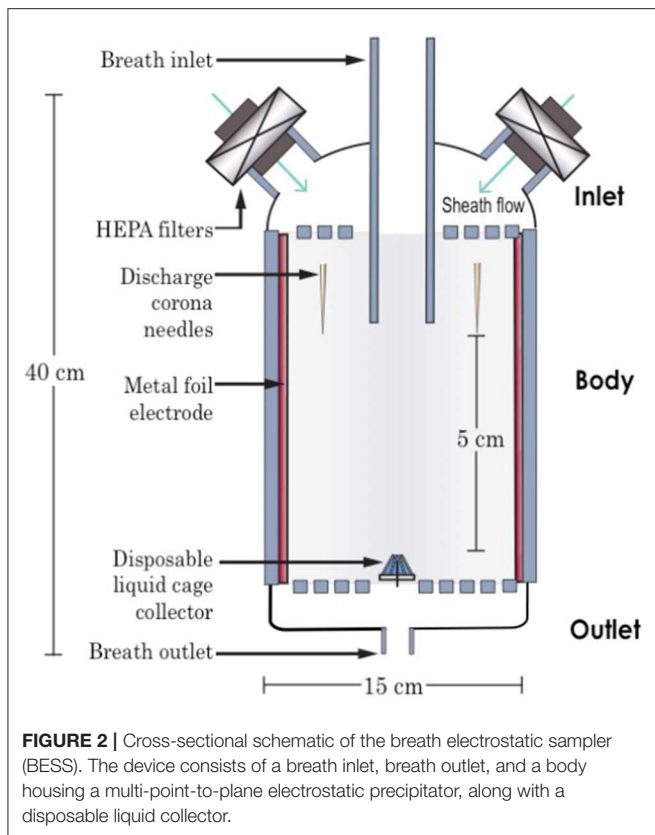
Study Design

We conducted the study at primary care centers in Antwerp, Belgium. We obtained ethical approval from the committee of the University Hospital Antwerp (UZA) and the University of Antwerp, Eudract number 2015-005724-26. Patients presenting with flu-like symptoms were referred to the study team by their family physician. After an explanation of the study, we asked the patients for their written consent, and three types of samples were collected: (1) a nasopharyngeal (NP) swab sample, (2) a breath aerosol sample using the BESS, and (3) a breath aerosol sample using a gelatin filter. Diagnosis was established from analysis of NP swabs. Filter collection was employed as a control for breath sampling, due to its widely accepted use and its excellent collection efficiency.

The BESS Electrostatic Sampler

The BESS is a multi-point-to-plane electrostatic precipitator contained within a hollow 3D-printed cylindrical housing (Figure 2). The multi-point discharge electrode is composed of





five tungsten needles placed cylindrically around the aerosol inlet. The collector electrode is composed of an electrically-connected 200 μL liquid droplet housed within a disposable 3D-printed cage collector, placed 5 cm downstream of the discharge needles. A third shielding electrode lines the internal wall of the cylindrical housing and is composed of a sheet of stainless steel. We applied a DC electrostatic potential of -7 , $+2.8$, and -4 kV, respectively, to the discharge, collector, and wall electrodes. The high electrical potential applied at the sharp needle induces a corona discharge which ionizes the surrounding air. These ions are attracted by electrostatic force toward the electrode of opposite electrical charge, here the liquid collector, creating an electrohydrodynamic wind. Airborne particles traveling through this ion stream become electrically charged by collision with the charged ions, thereby also experiencing the effect of electrical force toward the liquid collector electrode. The third shielding wall electrode serves the purpose of concentrating the electrical field line toward the collector electrode, while simultaneously avoiding uncontrolled electrostatic charging of the wall surface, which may otherwise attenuate the discharge and electrostatic precipitation onto the collector electrode. We fitted two disposable HEPA filters and a disposable mouthpiece at the inlet and connected the outlet to further HEPA filters and a vacuum pump. These extra inlets allow for creating a sheath flow, which help creating a more controlled airflow stream in the device, concentrating the exhaled breath in the center, and preventing direct contact between the discharge electrode and the exhaled breath.

Breath Sampling Protocol

To limit the experimental burden on the sick patients and minimize sample collection time, we restricted the breathing maneuver to five slow exhales. Breath aerosols were sampled first with the BESS, within a liquid collector loaded with a 200 μL droplet of universal transport medium (UTM) (COPAN, U.S.A.); and next with a 25 mm gelatine filter (SKC Inc.), placed inside its cassette holder. Please refer to **Supplementary Material** for details of the experimental setup and methods. We stored all samples at 4°C until the end of each day, after which we transferred them to the UZA hospital lab where we stored them at -80°C until analysis was performed.

Sample Analysis

We prescreened the diagnoses of all 20 patients by analyzing their NP swabs using the FTD Respiratory pathogens 21 plus panel (Fast Track diagnostics, Luxembourg), see **Supplementary Table 1** for a list of the pathogens and their limits of detection. Breath samples from BESS and filters were then analyzed using the FTD panel according the manufacturer's instructions.

RESULTS

Diagnosis via Nasopharyngeal Swab

Prescreening of 20 patients via their NP swabs revealed the following diagnoses: 1 with InfA, 5 with Inf B, 2 with Inf B and *S. aur*, 1 with CoOC43, 2 with CoOC43 and *S. aur*, 1 with CoHKU1 and adenovirus, 2 with rhinovirus, and 4 with no pathogens detected. The remaining two patients had positive samples from breath but not swab. Four patients had multiple diagnoses, i.e., multiple pathogens were detected from their NP swabs. Please refer to **Supplementary Table 1** for detailed Ct values. Unfortunately, after this point, breath samples from 12 patients were discarded due to a handling error. We tested the remaining eight patient breath samples using the FTD panel, see **Table 1**.

Analysis of Filter Samples

Analysis of BESS samples indicated seven patients who showed positive for *S. aureus*: 2 who had no diagnoses (BE110 and BE115); 3 diagnosed with *S. aureus* (BE116, BE117, and BE043), and; 2 diagnosed with HRV (BE047 and BE058). No viruses were detected.

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DISCUSSIONS

This pilot study provides a proof-of-concept for electrostatic sampling at the point-of-care and investigates this type of device for capturing exhaled aerosols, a previously unattempted

TABLE 1 | Results from the Fast Track Diagnostic respiratory pathogens 21 plus panel.

Patient ID	Swab	Breath		Agreement
		Filter	BESS	
BE110		CoHKU1	<i>S. aur</i>	
BE115			<i>S. aur</i>	
BE116	Inf B	HRV		
	<i>S. aur</i>		<i>S. aur</i>	+
BE117	Inf B			
	<i>S. aur</i>		<i>S. aur</i>	+
BE043	CoOC43			
	<i>S. aur</i>		<i>S. aur</i>	+
BE047	HRV		<i>S. aur</i>	
BE058	HRV	Co299E	<i>S. aur</i>	
BE064	CoOC43			

Samples were analyzed from nasopharyngeal swabs and exhaled breath, collected using two methods: filters and the BESS device. For each patient the pathogen detected in swab or breath is indicated. Empty cells indicate no pathogen detected. Agreement between swab and breath is indicated by a plus sign. Yellow cells indicate borderline positive results, where Ct > 38. Pathogens detected were: influenza B (Inf B), coronavirus CoOC43, coronavirus CoHKU1, coronavirus Co299E, rhinovirus (HRV), and *Staphylococcus aureus* (*S. aur*).

sampling method for breath. Despite the limited study size ($n = 8$) of patient samples tested, we discern a few potential trends. Combining the BESS with downstream qPCR analysis, we were able to non-invasively detect *Staphylococcus aureus* bacteria from the exhaled breath of patients. Additionally, filter-collected exhaled breath samples indicate capture of coronavirus and rhinovirus. From the ten positive breath samples detected, three were in agreement with their corresponding patient diagnosis, all captured using the BESS. These results indicate the BESS as a potentially non-invasive sampling device for bacterial detection. The 30% agreement between breath and swab samples is corroborated by Zheng et al. (2018), who find a 35% agreement between their breath samples and throat swabs [where analysis is made using the loop mediated isothermal amplification (LAMP) method].

Discrepancies

The capture of *S. aureus* from exhaled breath with the BESS device but not filters could be attributed either to low recovery efficiencies for *S. aureus* from the filters, as reported elsewhere (Chang and Wang, 2015); or to sources of error such as contamination during sample handling or collection. *S. aureus* is a known commensal organism, thus prone to spread through contact easily.

The size-dependent collection efficiency of electrostatic samplers, specifically their reduced efficiency for sub-micron particles (Parker, 2003), may explain the lack of virus capture by the BESS. *S. aureus* with size $\sim 1 \mu\text{m}$ (Monteiro et al., 2015) are thus more likely to be captured, rather than viruses whose sizes range from 20 to 500 nm. Future improvements could involve increased collection efficiency of the BESS

for submicron particles by integrating soft x-rays (Kulkarni et al., 2002) or a non-thermal plasma pre-charger (Thonglek and Kiatsiriroat, 2014). This will require further technical development and testing.

The differences in virus presence between the filter and the swab samples may indicate a more significant presence of viruses in the nasopharynx vs. airways. Unfortunately, we could not repeat the analysis due to the limited amount of sample available.

The differences in the presence of *S. aureus* between breath and swab samples may be attributed to either the previously mentioned contamination, poor swabbing techniques, or low bacterial loads present in the nasal passages vs. airways.

Limit of Detection

The presence of pathogens in breath aerosols were expected to occur in minimal amounts, based on previous investigations. Fabian et al. (2008) captured influenza from breath, detecting as few as 3–20 RNA cp/min of breathing; while Milton et al. (2013) demonstrated minimum 21 cp/30 min of breathing and 10 \times coughing. Thus, it is crucial to consider the limit of detection (LoD) of not only the analysis technique (qPCR in this work) but also the breath sampling methods (filters or the BESS in this work) to ensure detection of these low viral loads. The FTD panel assay used for sample analysis features an at-best LoD of 102–105 cp/mL, refer to **Supplementary Table 3** for LoDs of each pathogen. Minimizing the collection volume of the BESS would reduce sample dilution and deliver a potentially lower LoD. The BESS device, with a sample volume of 230 μL and assuming 100% capture efficiency, is potentially 6.5 times more sensitive than filters, which are diluted into 1.5 mL of buffer. Together, the BESS + FTD sampling+detection scheme offers a theoretical LoD of 23–104 copies while the filter + FTD scheme offers a theoretical LoD of 150–105 copies. Considering a viral load of Influenza in breath of 20 cp/min (Fabian et al., 2008), these schemes would require minimum 1 min breathing time into the BESS device, but 7 min of breathing time into filters, providing further motivation for considering the BESS device for sampling breath aerosols.

PCR-based assays, although relatively sensitive, might not form the best detection strategy. Alternative molecular methods could be considered, such as targeting nucleoproteins of virus capsids, which have a higher copy-number as compared with nucleic acids (Leirs et al., 2016) to push the limits of the technique and potentially yield an even lower LoD (Decrop et al., 2017).

Exhaled Virus Capture in Literature

Table 2 summarizes results from previous studies, which emphasize the minute amounts of pathogens present in exhaled breath (Fennelly et al., 2004; Huynh et al., 2007; Fabian et al., 2008; Stelzer-Braid et al., 2009; Lindsley et al., 2010; St George et al., 2010). Moreover, results can vary significantly depending on the day of illness onset, and from patient to patient. The substantial variation of breathing/coughing maneuvers and analysis methods utilized in these studies creates difficulties in comparing results and establishing conclusive outcomes.

TABLE 2 | Summary of previous studies demonstrating detection of exhaled pathogens.

References	Population	Pathogen	Collection method	Analysis technique	Maneuver	Results
Fennelly et al. (2004)	16 patients	<i>Mycobacterium tuberculosis</i>	Andersen impactors and sputum	Culture of agar	Coughing for 2 × 5 min sessions	3–633 CFU
Huynh et al. (2007)	20 patients	HRV, RSV, Influenza, pneumonia	Nasal mucus and Masks	PCR	20× cough and 20 min breathe	45% pos
Fabian et al. (2008)	12 patients, 2 subjects	Influenza	Rapid flu test, Teflon filter and OPC	PCR, OPC	20 min breathing	33% pos, < 48–300 cp/filter and < 3.2–20 cp/minute of breathing
Wainwright et al. (2009)	28 patients	<i>Pseudomonas aeruginosa</i> and gram-neg bacteria	Andersen impactors	Culture and PCR	5 min coughing	89% pos, 0–13 485 CFU
Stelzer-Braid et al. (2009)	50 patients	Influenza, RSV, pneumonia, picorna, HRV	Nasal mucus, masks	PCR and cell culture	10 min breathing, 10 min talk, 1 min coughing	50% pos, 2000, 200 TCID50 /mL from nasal breathing, and 200 TCID50/mL from cough
St George et al. (2010)	19 volunteers	Influenza	EBC, np swab	culture and PCR	10 min tidal breathing	1 pos, 37.1 Ct value
Lindsley et al. (2010)	58 patients	Influenza	NP swab, NIOSH cyclone, biosampler	PCR and plaque assay	6× coughing	38 patients coughed flu. 15 cp/cough.
Fabian et al. (2011)	77 subjects	HRV	Gelatin filter, OPC, nasal lavage	PCR and OPC	Coughing, swallowing and rapid exhale	13–33% pos from tidal breathing, 66% pos from coughing
Xu et al. (2012)	7 patients	<i>Sphingomonas paucimobilis</i> , <i>Kocuria</i>	EBC (impaction)	Culturing, DNA stain, SEM and PCR	1 min exhale, 2 min exhale, 3 min exhale, 4 min exhale	1 influenza pos, 7000 CFU/m3 bacteria
Milton et al. (2013)	38 volunteers	Influenza	NP swab, masks	PCR and culture	30 min breathing, 10× coughing	43% pos
Gralton et al. (2013)	12 adults, 41 children	Influenza, RSV, HRV, pneumonia	Impactor	PCR	2–5 min breathing	39% pos
Hatagishi et al. (2014)	56 patients	Influenza	Gelatin filter and throat swab	PCR and plaque assay	20× coughing	41% pos, <10–2240 cp/cough
Lindsley et al. (2016)	61 patients	Influenza	SKC biosampler	Viral replication assay	Cough and exhale 3 times	86% pos
Zheng et al. (2018)	100 patients	13 bacteria	EBC, throat swab	LAMP	5 min continuous exhale	30% pos
Santarpia et al. (2020)	11 isolation rooms	SARS-CoV-2	Environmental air and surface samples post patient isolation onto gelatin filters	RT-PCR	5–18 days of isolation	63.2% in room air (2.86 copies/L of air) 66.7% outside the rooms in the hallways

The sparse datasets available on pathogen loads in breath, differences in breathing maneuvers, and the small population sizes in the studies emphasize the need for further in-depth and large-scale clinical studies to understand the pathophysiology of respiratory pathogens.

The aim of our study was not to demonstrate the potency of direct pathogen sampling for exhaled breath, but rather to explore the clinical potential of our approach. Our results and that of others indicate that exhaled breath as a sampling medium is yet to be better explored. With the emergence of novel technologies enable sampling with higher efficiencies, a better understanding of the benefits of breathing maneuver, and more sensitive detection technologies, we may discover areas in which exhaled breath may represent a useful sample source for non-invasive diagnostic testing. However, more work is yet needed before to be able to draw such conclusions.

CONCLUSIONS

We demonstrate, for the first time, the use of electrostatic-based methods for the capture of breath aerosols from patients at primary care centers. Our BESS device, particularly adapted for point-of-care settings, demonstrated capture of *S. aureus* from the breath aerosols of patients with a 30% agreement to patient diagnosis, whereas filters captured exhaled viruses. Discrepancies were observed between the two breath sampling methods and between breath and nasopharyngeal swab samples, indicating the need for further investigations. A review of previous studies indicates the exceptionally low pathogen loads available in exhaled breath, indicating the need for further investigations and large-scale studies. To address this low pathogen load, we suggest optimizing patient breathing maneuvers and targeting viral proteins, rather than nucleic acids, to achieve a sensitive sampling-detection scheme.

Breath sampling presents a promising non-invasive solution for studying transmission or pathophysiology of lower respiratory tract infections; and further research is required to potentially allow reliable detection of respiratory pathogens using electrostatic breath samplers.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Commissie voor Medische Ethiek UZA/UAntwerpen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmech.2020.00040/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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