Clinical Application of Volatile Organic Compound Analysis for Detecting Infectious Diseases

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SUMMARY	462
INTRODUCTION	462
ANALYTICAL METHODS FOR DETECTION OF VOLATILE METABOLITES	463
Sample Collection	463
Chemical Analysis and Instruments	463
Chemical Sensing and Sensor Devices	464
VOCs AND DIAGNOSIS OF INFECTIOUS DISEASES	466
Respiratory Infections	466
<i>Mycobacterium tuberculosis</i>	466
<i>Pseudomonas aeruginosa</i>	468
<i>Aspergillus fumigatus</i>	469
Gastrointestinal Infections	469
Urinary Tract Infections	469
Other Infections	470
CONCLUSIONS AND PERSPECTIVES	470
REFERENCES	472
AUTHOR BIOS	475

SUMMARY

This review article introduces the significance of testing of volatile organic compounds (VOCs) in clinical samples and summarizes important features of some of the technologies. Compared to other human diseases such as cancer, studies on VOC analysis in cases of infectious diseases are limited. Here, we have described results of studies which have used some of the appropriate technologies to evaluate VOC biomarkers and biomarker profiles associated with infections. The publications reviewed include important infections of the respiratory tract, gastrointestinal tract, urinary tract, and nasal cavity. The results highlight the use of VOC biomarker profiles resulting from certain infectious diseases in discriminating between infected and healthy subjects. Infection-related VOC profiles measured in exhaled breath as well as from headspaces of feces or urine samples are a source of information with respect to disease detection. The volatiles emitted in clinical matrices may on the one hand represent metabolites of the infecting pathogen or on the other hand reflect pathogen-induced host responses or, indeed, a combination of both. Because exhaled-breath samples are easy to collect and online instruments are commercially available, VOC analysis in exhaled breath appears to be a promising tool for noninvasive detection and monitoring of infectious diseases.

INTRODUCTION

Emerging advances in analytical technologies for detecting and measuring volatile organic compounds (VOCs) in clinical matrices have generated increasing interest for their use in evaluating the diagnostic potential of VOCs for different diseases (1–3). VOCs represent a wide range of stable chemicals, volatile at ambient temperature (may emit odors), and are detectable in exhaled

breath, urine, feces, and sweat (3, 4). Testing for volatile biomarkers in clinical samples offers an option for developing rapid and potentially inexpensive disease screening tools. Most of the studies on volatile biomarkers have been carried out on exhaled-breath samples (5–11), although other clinical matrices, such as urine (12) and feces (13), have also been investigated. Analysis of breath samples for testing of volatiles can be performed frequently in follow-up studies, which may reflect disease progression and be helpful in monitoring therapeutic intervention. Moreover, breath tests (BTs) are noninvasive and thus suitable for critically ill patients (in intensive care units) and small children. Furthermore, they have been proven to be useful for diagnosing a broad range of diseases, including diabetes (14–16), gastrointestinal and liver diseases (17), lung disorders (10, 18), different types of cancer (19–22), and infections (3, 23, 24).

VOCs are either inhaled or absorbed through the skin from the environment and subsequently contribute to exhaled breath. Furthermore, they present themselves as endogenous products of physiological/metabolic body processes or products of various microbial pathogens/commensals, or they are produced by the host in response to microbial infections, e.g., during the inflammatory response. It is believed that VOCs are transported from different organs via blood to the lungs and subsequently excreted from there by diffusing across the pulmonary alveolar membrane and exhaled via breath (25). Assessment of endogenous VOCs can

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provide insights into healthy and diseased metabolic states, whereas the detection of exogenous compounds suggests exposure to a drug or compound associated with environmental or occupational exposure (26).

The introduction of new analytical approaches and technological developments in instrumentation has enabled the detection of low concentrations of VOCs in clinical samples such as exhaled breath, urine, and feces of patients and has allowed us to predict those which are found to be distinctive (based on qualitative and/or quantitative variations) compared to healthy individuals or are linked to a disease state (27–32).

To date, limited progress has been made in the identification of body-derived endogenous VOC profiles associated with infectious diseases that could be used as disease biomarkers. The objective of this article is to review emerging studies focusing on the utility of VOC analysis as a diagnostic tool for detecting and monitoring infectious diseases in a clinical setting. The extensive literature concerning the use of VOC analysis for *in vitro* identification and classification of microorganisms, including pathogens, is already the subject of various review articles (2, 12, 32–35) and will not be addressed here. Thus, after a brief introduction to some of the newly developed analytical technologies used for VOC analysis in clinical matrices, we focus on findings of studies which have used these novel approaches for investigating the potential of disease-related VOC biomarkers or for generating marker profiles enabling detection of underlying infectious diseases.

ANALYTICAL METHODS FOR DETECTION OF VOLATILE METABOLITES

Over the last 2 decades, significant advances have been made in sample collection and preconcentration methods, including the development of a wide range of analytical procedures for the identification of VOCs in exhaled breath and other clinical matrices. Details have been extensively reviewed in the literature (5, 6, 27–32) and are beyond the scope of the present review. Essential features of important methodologies are briefly summarized below.

Sample Collection

For collection of breath samples, a distinction is made between “dead-space air” volume (ca. 150 ml air) contained in upper respiratory airways (mouth/pharynx) that is not involved in gas exchange and the next 350 ml of alveolar air from deeper lung regions containing blood-based VOCs, which is subjected to analysis (5). Several techniques/devices have been used for collection and capturing of volatiles from clinical samples before further analysis. The sample can be delivered directly into the measuring instrument in a single step or first collected and stored in a container, usually a polymer bag or canister. The use of disposable mouth pieces and bacterial filters prevents the risk of patient-to-patient contamination. Sampling devices employ in-line spirometers to control breath volume. For the headspace sampling technique, it is necessary that the sample forms an equilibrium within a headspace over the sample matrix, which is coseparated from the ambient environment inside a bag such as Tedlar bags or stainless steel electropolished canisters (36, 37). Because VOCs contained in breath or headspace samples (liquid/solid) are present at very low levels, they require capturing, which can be achieved by using chemical trapping, sorbent trapping, cold trapping, or condensate trapping techniques (38). This is then followed by thermal desorption (commercially available automatic

desorption devices) before they are analyzed for VOC content. The advantages and disadvantages of these techniques have been described in detail elsewhere (38).

Preconcentration of breath volatiles achieved via solid-phase microextraction (SPME) can be automated for adsorption and desorption purposes (39). SPME followed by gas chromatography-mass spectrometry (GC-MS) (SPME-GC-MS) provides a sensitive tool to identify and quantify VOCs in trace amounts (6). A more recently developed methodology which integrates both sampling and preconcentration in one step is membrane extraction with a sorbent interface (40).

Exhaled-breath condensate (EBC) is a promising technique in which aerosolized microdroplets from the lower respiratory tract are captured by directing the exhaled air through a cooling/freezing device, which results in the accumulation of EBC in the chamber. EBC is composed mainly of condensed water vapor and a very small fraction of droplets containing a range of both volatile and nonvolatile components (e.g., cytokines, leukotrienes, and DNA, etc.). In general, EBC trapping is inefficient because of variable dilutions of microdroplets which carry VOCs and also poses the risk of contamination with nonvolatile components present in the aerosol (24, 41). Recommendations to standardize the collection of EBC have been made by the American Thoracic Society (ATS)/European Respiratory Society (ERS) task force on EBC (42).

Chemical Analysis and Instruments

Basic analytical tools used for detection of volatiles include GC, MS, GC-MS, chemiluminescence, optical absorption spectroscopy systems, electronic noses, and different types of gaseous sensors. The earliest method used for volatile detection in clinical samples was GC coupled to a suitable detector. The class of volatiles detected by GC depends on the type of detector (plasma ionization, plasma photometric, photoionization, and electron capture detectors) used. It has quickly become apparent that the identification of VOC profiles that could serve as biomarkers for a particular disease necessitates the use of appropriate technologies/instruments which can perform this type of analysis in clinical matrices.

There are several analytical procedures that are linked to GC, including flame ionization detection (GC-FID) and ion mobility spectrometry (GC-IMS), which enable sensitive detection of VOCs (27). GC-FID, which is widely used for breath analysis, detects organic compounds with high sensitivity, a linear response, and low background noise (43). GC-IMS, which enables quantification of separated VOCs, is based on separation of ions in relation to their gas phase mobilities. The ion mobility spectrometer is suitable for breath molecular profiling in biomarker discovery (44).

Nonoptical methodologies for VOC measurement also include a combination of mass spectrometers and selected ion flow tubes as well as the method of proton transfer reaction mass spectrometry (PTR-MS). Both methods work online and do not require preconcentration and separation steps. PTR-MS enables volatile monitoring (selective for oxygen-containing compounds) according to their mass-to-charge ratios; however, using this method, a chemical identification is not possible (45). Therefore, the PTR ionization technology can be coupled to a time of flight mass spectrometer (PTR-TOF), thereby making an online breath analysis possible. A prototype instrument for PTR-TOF with these capabilities has been developed (28). A limitation of the PTR-MS

FIG 1 Flow chart showing a typical study design. PLS-DA, partial least-squares model for discriminant analysis; MESI, membrane extraction with sorbent interface; SPME, solid-phase microextraction; NIST, National Institute of Standards and Technology; PCA, principal component analysis; Neg, negative; Pos, positive; VOCs, volatile organic compounds; GC-MS, gas chromatography-mass spectrometry.

method is the fact that each measured mass with its corresponding atomic mass unit (amu) (range, 21 to 229 amu) can represent a combination of various substances with the same amu, making it difficult to identify the specific substance. Another methodology with potential for clinical use is selected ion flow tube mass spectrometry (SIFT-MS), which provides real-time quantification of several VOCs simultaneously and is suitable for online, noninvasive breath analysis for clinical diagnosis and therapeutic monitoring. A relatively small SIFT-MS-based analytical instrument (Profile 3) has been developed for VOC-based breath testing and is suitable for routine use in a clinical setting (46).

Optical spectroscopic methods (laser absorption spectrometry) are also useful for the detection and quantification of specific gases in a mixture (30). These methods are highly sensitive and selective and can be connected to different types of spectroscopic sensors, such as acoustic wave sensors, sensors with conductive polymers, and other sensors (47) for detecting specific gases (30).

Considerable progress has been made in the development of automatic devices for the collection and preconcentration of sam-

ples from clinical matrices as well as for VOC separation and detection purposes. However, accepted criteria for the standardization and normalization of the methodologies as well as for data processing, statistical analyses, and evaluation in order to be clinically useful and comparable across independent studies are lacking (6, 10). Typical steps involved in a GC-MS-based study design for the selection of disease-specific VOC breath biomarkers/profiles in exhaled-breath samples are depicted in Fig. 1.

Chemical Sensing and Sensor Devices

Gas-based chemical sensing devices, frequently denoted electronic noses (e-noses), have been used successfully for the analysis of volatile metabolites in clinical specimens (32, 48, 49). They are designed on a principle that electronically mimics the mammalian olfactory system and utilize a variety of technologies. The most commonly used e-noses employ nonselective chemical vapor sensor arrays, which are combined with pattern recognition software, based on a statistical pattern-matching algorithm, and a classification technique. This technology records distinct patterns in re-

TABLE 1 Advantages and disadvantages of some of the methods currently used for VOC analysis in clinical matrices

Method	Advantage ^a	Disadvantage
Gas chromatography with mass spectroscopy	Identification of unknown VOCs and profiling possible Sensitivity in the ppb range Reproducible	System immobile Slow Preconcentration needed Quantification requires known compounds Real-time measurements not possible Expensive Not suitable for clinical use
Ion mobility spectrometry	No preconcentration needed Sensitivity in the ppm range Mobile system Low cost Suitable for clinical use	VOC chemical identification and profiling not possible Real-time measurements not possible
Selected ion flow tube mass spectrometry (e.g., Profile 3 instrument)	Measures VOCs in real time Fast Potential for online testing VOC measurement in headspace (serum/urine) possible Sensitivity in the ppb range Mobile system	VOC chemical identification and profiling not possible
Proton transfer spectrometry	No preconcentration needed Real-time measurements and online monitoring possible Sensitivity in the ppb range	VOC chemical identification and complete profiling not possible
Various chemical sensor matrix platforms/e-noses	Easy to use Cheaper Portable Rapid Suitable for bedside use Sensitivity in the ppb range	Sensors not designed for single VOCs Devices not compatible with each other

^a ppb, parts per billion; ppm, parts per million.

sponse to different VOCs present in a gas mixture of unknown composition, excluding the need for chemical separation or identification of individual components (50).

The most commonly used data processing methods are principal component analysis, cluster analysis, discriminant function analysis, genetic algorithms, and neural network algorithms. Some e-noses are used to analyze breath samples by detecting odor and transduce the vapors into electrical signals, which are then analyzed by pattern recognition software (31).

Increasing attention is being focused on different types of chemical sensor matrix platforms, which include sensors based on conducting polymers and metal oxides, surface acoustic wave sensors, and optical sensors (32). A notable development is the use of nanotechnology (gold nanoparticles, nanowires, and nanotubes) for the design of VOC sensors (51, 52). The output from sensor devices may be a change in color, fluorescence, conductivity, vibration, or sound following the interaction of the VOC compound with the sensor surface. Typically, these devices consist of a detector chip with one or more individually addressable detection elements, each capable of being independently functionalized to detect volatile target analytes (with partial and overlapping specificities for certain classes of chemicals rather than single compounds) in the vapor headspace over a sample (50). The partial specificity accounts for the generation of a unique signature of the

sample where the acquired sensor data (quantitative or semiquantitative) can be combined with artificial-intelligence approaches and Web-based knowledge systems in a clinical diagnostic setting (53).

The e-nose devices can be tailored for specific applications using sensor arrays, which should be able to detect all or individual volatile components included in the specific predetermined VOC profile. A further development in this direction is the Z-nose. The Z-nose is a device based on acoustic technology which consists of a gas chromatographic detector and a direct column heating system (54, 55). The benefits of gaseous chemical-sensing devices over other techniques are that they are easy to use for point-of-care (POC) testing; however, the drawback is that they provide only a collective response of the sensors to the analytes being sensed in a mixture without identifying specific chemical compounds. Considerable literature dealing with e-nose technologies and their various applications in diverse fields, including identification of *in vitro*-cultured pathogens, is available and has been reviewed extensively elsewhere (31, 32, 49). The explicit focus of this review is those studies which have evaluated the direct disease detection performance of e-nose devices in relation to infections. A list of some of the methods currently used in breath analysis and their advantages and disadvantages is provided in Table 1.

VOCs AND DIAGNOSIS OF INFECTIOUS DISEASES

Infectious diseases are the major cause of death in many countries, and an early diagnosis may initiate appropriate antimicrobial therapy for efficient patient management. Conventional microbiological procedures are time-consuming and require invasive procedures for obtaining clinical specimens. There is a need to develop rapid, cheap, convenient, and accurate tests for the diagnosis of infectious diseases, to initiate rapid pathogen detection and subsequent specific treatment. The identification of VOC biomarkers for infections is the focus of considerable clinical research interest mainly because of their disease-detecting potential.

The use of unusual body smell (odor sensation/human olfaction) to diagnose diseases was practiced by ancient physicians (1, 31). In recent years, several reports have documented that dogs can be trained to detect various types of cancers based on cancer-specific odors (volatiles) from a patient's urine, tumor tissue, or breath (56). Furthermore, African giant pouched rats have been shown to detect *Mycobacterium tuberculosis*-positive sputum samples (57).

Various microorganisms, including human pathogens, are known to produce characteristic volatile metabolites (58), which can be detected through headspace screening of bacterial cultures (59). VOC "fingerprinting" or "smell printing" has been used for *in vitro* identification, classification, and discrimination of microorganisms (33, 59, 60). The recent availability of highly sensitive detection technologies linked to GC-MS instruments combined with novel gas sensor devices (e-noses) has provided necessary tools to identify disease-specific VOC biomarkers in clinical matrices.

In the following sections, we review studies which have employed new analytical approaches to identify VOC biomarkers associated with infectious disease states. The reports deal with certain respiratory, gastrointestinal, urinary tract, and nasal infections.

Respiratory Infections

The majority of published reports have centered on VOC analysis from exhaled breath for establishing the etiologic diagnosis of respiratory infections. There is limited literature available dealing with the assessment of urine or feces for identifying potential VOC biomarkers for other infections. The studies reviewed in this section include pulmonary infections caused by pathogens such as *M. tuberculosis*, *Pseudomonas aeruginosa*, and *Aspergillus fumigatus*.

***Mycobacterium tuberculosis*.** Tuberculosis (TB), an important infectious disease caused by the bacterium *M. tuberculosis*, is responsible for considerable morbidity and mortality worldwide (61). Currently available tests for the rapid detection of *M. tuberculosis* are inadequate, and there is an urgent need for improvements in TB diagnosis and for new methods to determine the efficacy of treatment, particularly in developing countries (62–64). The strategy of developing tests based on TB-associated VOC biomarkers offers substantial advantages over other approaches in that such tests are simple, noninvasive, and suitable for adaptation in the form of POC testing devices (e.g., e-noses).

As early as 1923, a review article entitled "Aroma-Producing Microorganisms," which appeared in the *Journal of Bacteriology*, reviewed the subject of odor association with microorganisms and listed, among others, *M. tuberculosis*, known for its foul smell (65).

Reportedly, the Greek physician Hippocrates practiced pouring human sputum on hot coal to diagnose TB on the basis of the emanating foul odor (cited in reference 64).

In more recent years, an increasing number of studies have focused on the application of sensor array-based devices (e-noses) to obtain volatile smell prints in order to discriminate between TB patients and noninfected controls (66–68). An earlier study used electroconductive sensor-based e-nose technology for demonstrating the discrimination of *M. tuberculosis* from other bacterial species in sputum samples (66). Next, an e-nose comprising 14 conductive polymers was employed to examine the headspace of sputum samples from 55 culture-positive TB patients and 79 non-TB individuals. Based on artificial neural network analysis of the data, the e-nose correctly predicted 89% of culture-positive patients, with a specificity and sensitivity of 91% and 89%, respectively, compared to culture evaluation (67). Later, this same group compared sputum headspace samples from Ziehl-Nelson stain-negative and culture-positive TB patients using 2 different e-nose devices. The concluding data for the ENRob e-nose showed a sensitivity and specificity of 68% and 69%, respectively (accuracy, 69%), and the data for the ENWalter device showed sensitivity and specificity values of 75% and 67% (accuracy, 69%), respectively (68). From this study, it was concluded that commercially available e-noses are not yet accurate enough to differentiate TB patients from non-TB individuals. The sensitivities of commercially available e-noses to different chemical groups and concentrations vary with the particular sensor technology used in the fabrication of the devices (20).

GC-MS analysis of headspace VOCs from *in vitro*-cultured *Mycobacterium* species revealed several metabolites of nicotinic acid, such as methyl phenyl acetate, methyl p-anisate, methyl nicotinate, and o-phenylanisole, which were considered specific for *M. tuberculosis* complex strains (69). These compounds represent derivatives of nicotinic acid, which can also originate from tobacco or food materials. In fact, a comparison of the analysis of breath samples from nonsmoking patients with TB and those without TB showed a striking difference in levels of nicotinic acid between the two groups (69). In a subsequent study, this same group reported the detection of methylnicotinate, a metabolite of nicotinic acid, in the exhaled breath of TB patients, achieving a sensitivity of 84% but a low specificity of 64% among patients with smear-positive TB (70).

In a separate study, GC-MS recorded 130 identifiable VOCs from the headspace of cultured *M. tuberculosis*, of which the 10 most abundant VOCs, listed in Table 2, were selected (71). GC-MS analyses were performed on exhaled-breath samples of 42 patients with clinical suspicion of pulmonary TB (sputum culture positive, $n = 23$; negative, $n = 19$) and 59 healthy controls. Breath samples of all patients with TB contained the *M. tuberculosis*-associated markers naphthalene, 1-methyl- and cyclohexane, 1,4-dimethyl-, which were identical, as well as VOCs which were structurally similar to those observed in *in vitro* cultures (Table 2). In addition, they also revealed increased markers of oxidative stress (C_4 to C_{20} alkanes and monomethylated alkanes). Fuzzy-logic and pattern recognition analysis of the data identified patients with a positive sputum culture with sensitivities and specificities of 95.5% and 78.9% and 82.6% and 100%, respectively (71). This same group extended the investigations to a multicenter international study in which breath samples collected in portable breath collection devices from a total of 226 symptomatic high-

TABLE 2 Breath VOC biomarker profiles selected^a for detection of pulmonary TB patients contain VOCs identical and/or structurally similar to those secreted in mycobacterial cultures *in vitro*^b

VOC profile		
<i>M. tuberculosis</i> (<i>in vitro</i> culture)	Breath (TB) ^c	Breath (active TB)
Naphthalene, 1-methyl- <i>trans</i> -Anti-1-methyl-1-decahydronaphthalene	Naphthalene, 1-methyl- ^d	Naphthalene, 1-methyl-
Cyclohexane, 1,4-dimethyl-	Cyclohexane, 1,4-dimethyl- ^d Cyclohexane, 1,3-dimethyl- <i>trans</i> - Cyclohexane, 1-ethyl-4-methyl- <i>trans</i> -	Cyclohexane, hexyl-
3-Heptanone	Heptane, 3-ethyl-2-methyl-	
Heptane, 2,2,4,6,6-pentamethyl-		Heptane, 2,2,4,6,6-pentamethyl-
Benzene, 1-methyl-4-(1-methylethyl)-	Benzene, 1,2,3,4-tetramethyl- Benzene, 1,4-dichloro-	Benzene, 1,3,5-trimethyl-
Butanal, 3-methyl- 2-Hexene	2-Butanone	1-Hexene, 4-methyl
Methylcyclododecane 3,5-Dimethylamphetamine		

^a The profiles selected also included biomarkers of oxidative stress (alkanes and alkane derivatives).^b See references 71 to 73.^c Fuzzy-logic discriminators of infection.^d Denotes VOCs identical to those detected in *in vitro* culture; the others are structurally similar but not identical.

risk patients (United States, Philippines, and United Kingdom) were investigated by using automated thermal desorption GC-MS (72). Breath volatiles contained components related to oxidative stress, such as alkanes (e.g., tridecane) and methylated alkane derivatives (4-methyl-dodecane), as well as *in vitro*-defined VOCs of *M. tuberculosis* origin (e.g., cyclohexane, benzene, decane, and heptane derivatives). This VOC pattern in the breath of patients with active TB identified *M. tuberculosis* infection in this randomly selected population with an overall accuracy of 85.5%, as revealed by C-statistic values, compared to diagnosis based on sputum culture, microscopic smear, chest radiograph, and clinical symptoms (72). In a similar study, a 6-min breath testing system for POC use (Breath Link) was applied to investigate 279 individuals at 4 centers in 3 different countries representing the Philippines, the United Kingdom, and India (73). Using multiple Monte Carlo analysis, a set of biomarkers corresponding to previously reported biomarkers of active pulmonary TB were selected. Multivariate predictive algorithm analysis of data from 251 subjects (130 with active TB and 121 controls) allowed correct detection of active pulmonary TB with a sensitivity of 71.2%, a specificity of 72%, and an overall accuracy of 80% (73).

Notably, *M. tuberculosis*-associated breath volatile metabolites detected in active pulmonary TB were structurally similar but not identical to those generated from *in vitro* cultures (73). The results suggest the feasibility of distinguishing active pulmonary TB from nonactive TB by breath VOC patterns. 1,3,5-Trimethylbenzene was identified in active pulmonary TB, whereas in the nonactive stage, 1,2,3,4-tetramethylbenzene was identified (72).

Breath samples from 50 patients with suspected TB and 50 non-TB patients from South Africa (region of endemicity) were analyzed by using GC-MS. A VOC profile consisting of 7 compounds (dodecane, 3-heptafluorobutyroxy-pentadecane, 5-hexonic acid, 1-hexanol, 2-ethyl-, tetradecanoic acid, octanal, and an unknown compound) could differentiate between TB and non-TB breath samples with a sensitivity of 72%, a specificity of 86%, and an accuracy of

79% compared with culture. A validation set of 21 TB and 50 non-TB subjects provided a sensitivity of 62%, a specificity of 84%, and an accuracy of 77% (74). None of the 7 discriminating VOCs employed in this study were identical to those identified in the headspace of *M. tuberculosis* cultures *in vitro*, which led the authors to conclude that they represent host response VOCs (74). Preliminary results of a study have identified a molecular signature of seven metabolites which could be useful in differentiating active TB, i.e., sputum microscopic test positive, from unexposed healthy (skin test-negative) subjects (R. Nanda, unpublished data).

Current studies suggest that pulmonary tuberculosis alters VOC composition in breath, because *M. tuberculosis* and oxidative stress resulting from infection both generate distinctive VOCs. Apart from TB, oxidative stress is also associated with various clinical entities and chronic inflammatory diseases (14, 75, 76). Oxidative stress occurs due to either the increased production of radical oxygen species (ROS) or a depletion/decrease of cellular antioxidant reserves, thereby creating an imbalance between host cell pro- and antioxidant levels (43). ROS are generated from molecular oxygen via electron transfer reactions and include superoxide ion, hydrogen peroxide, hydroxyl radical, and singlet oxygen (reviewed in reference 77). These ROS can damage cell membranes by peroxidating polyunsaturated fatty acids (lipid peroxidation), resulting in the generation of several breakdown products, including alkanes and methylated alkanes, which are excreted in the breath with altered composition and concentration and can be monitored as biomarkers of oxidative stress (for details, see reference 43).

Reportedly, GC-MS coupled to a headspace sampler can be used to distinguish VOC profiles in urine from TB patients and healthy controls (78). Data processing using principal component analysis and partial least-squares/discriminant analysis revealed a panel of 5 selected marker compounds (alpha-xylene, isopropyl acetate, 3-pentanol, dimethylstyrene, and cymol), which enabled discrimination between TB-infected and healthy individuals with

an accuracy of 98.8% (area under the curve [AUC] of 0.988). These compounds could also discriminate TB patients from patients with lung cancer and chronic obstructive pulmonary disease (COPD). The results offer the possibility of diagnosing TB on the basis of VOC signals in the urine of patients and could potentially be used in a clinical setting (78).

In a domestic animal model (goats) of chronic intestinal TB (*Mycobacterium avium* subsp. *paratuberculosis*), differential ion mobility spectrometry was used to analyze VOC patterns in both the breath and headspaces of feces from infected and noninfected animals (79). The VOC pattern data obtained from both breath and feces could discriminate *M. avium*-infected from noninfected animals, although the discriminatory attributes were stronger in breath than in feces. Interestingly, those authors reported quantitative differences (increase or decrease) in VOC detection in relation to infection (79). A recent investigation in cattle employed GC-MS to select 2 pathogen-related VOCs in the breath of *Mycobacterium bovis*-infected animals and 2 VOCs associated with healthy noninfected animals (80). These candidate VOC biomarkers (cyclohexane and pentadecane) were utilized for designing an array of sensors based on nanotechnology for detecting *M. bovis*-infected cattle. In field settings, this device identified all the infected animals and detected probable cases of infection among 21% of noninfected animals (80).

Reportedly, VOCs in headspaces of serum samples of cattle infected with *Mycobacterium avium* subsp. *paratuberculosis* can be used to discriminate infected from healthy cattle by using an e-nose device (81). Similarly, analysis of VOCs in headspaces of serum samples detected with an e-nose enabled discrimination of *M. bovis*-infected cattle and badgers from healthy animals (82).

***Pseudomonas aeruginosa*.** *Pseudomonas aeruginosa* is an important pulmonary pathogen, particularly in children with cystic fibrosis (CF), which is a genetic disease caused by mutations of the gene encoding the CF transmembrane regulator (83). Lungs of CF patients are either colonized or infected with this opportunistic bacterium, which results in an unfavorable prognosis of the course of the disease (83, 84). Because CF is a genetic disease, infection or colonization with *P. aeruginosa* is unique in the context that there is no underlying immune defect involved which predisposes the host to infections in general. Early detection of this bacterium is crucial for initiation of appropriate therapy and is therefore of high clinical relevance.

Several studies have reported the detection of high concentrations of hydrogen cyanide (HCN) in the headspace samples of *in vitro* cultures of *P. aeruginosa* strains (85–88). The production of HCN by *P. aeruginosa* is considered to be under specific genetic control (86), and its expression is determined by specific growth conditions, in particular oxygen levels (85).

HCN has been classified as a key marker for the presence of *P. aeruginosa* in the headspace of sputum cultures from children with CF (87). Elevated levels of HCN were detected by SIFT-MS in exhaled-breath samples from 16 children with CF compared to samples from 21 children with asthma (88). Notably, low levels of HCN have been detected in the oral cavities (89–91) and exhaled breath of healthy individuals (91). Importantly, HCN has been identified in the headspace of *in vitro*-cultured *Helicobacter pylori* strain NCTC11637 (92) and notably also in the breath of patients infected with this bacterium (92, 93). Thus, the detection of an identical biomarker(s) can indicate a different disease caused by an unrelated pathogen.

Besides HCN, the production of methyl thiocyanate has been reported in the headspace of *P. aeruginosa* cultures (28 out of 36 strains), as monitored by SPME-GC-MS and real-time SIFT-MS quantification methods (94). Methyl thiocyanate was also identified with the aid of SIFT-MS in the breath samples of 28 children with CF. The authors of this study noted that in order to determine the clinical relevance of this VOC marker for *P. aeruginosa* infection, a large clinical investigation is necessary (94). A breath test involving disease-related VOC biomarkers could be a suitable tool for noninvasive diagnosis of *P. aeruginosa* infections (88).

2-Aminoacetophenone (2-AA) represents another *P. aeruginosa*-associated volatile compound which has been proposed as a potential marker for detecting *P. aeruginosa* in culture (95–98). This volatile compound, which accounts for the grape-like odor of *P. aeruginosa* cultures, is consistently produced by *P. aeruginosa* strains (95–98) and is detectable in the headspace of *P. aeruginosa* cultures by GC-MS (98) and SPME-GC-MS (99). 2-AA has also been detected via SPME-GC-MS in the exhaled breath of 15/16 patients colonized with *P. aeruginosa* (93.7%), compared to 5/17 (29%) healthy controls and 4/13 (30.7%) CF patients not colonized with *P. aeruginosa*. The calculated sensitivity and specificity of this test compared to *P. aeruginosa* culture results were 93.8% and 69.2%, respectively. It was concluded that 2-AA may serve as a potential biomarker of *Pseudomonas* infection and/or colonization in CF patients (99). The origin of very low levels of 2-AA detected in a small proportion of healthy controls and subjects with CF (without evidence of *P. aeruginosa* upon sputum sampling) was unexplained (99). A subsequent study revealed the presence of 2-AA in a variety of foods, and consequently, the consumption of such foods may lead to a false-positive breath test for 2-AA, and improved breath sampling procedures have been recommended in order to avoid the effects of confounding factors on test results (100).

There is accumulating evidence that *P. aeruginosa* infection may be better detected by using a VOC biomarker profile rather than by using the single-biomarker approach. An ion mobility spectrometer coupled with a multicapillary column was used to examine the exhaled breath of 53 individuals, including 24 individuals infected or colonized with *P. aeruginosa* and 29 healthy controls. Of a total of 224 signals recorded, 21 enabled discrimination between the healthy and *P. aeruginosa*-infected groups with sensitivity and specificity values of 89% and 77%, respectively, and positive and negative predictive values of 83% and 86%, respectively (101). In a different methodological approach, breath samples from 105 children, comprising 48 children with CF and the respective controls, were examined for VOC profiles using GC-time of flight-mass spectrometry (GC-TOF-MS) (18). On the basis of 14 VOCs, it was possible to correctly identify CF patients with ($n = 23$) or without ($n = 17$) positive *P. aeruginosa* cultures with sensitivity and specificity ranges of 34 to 100% and 29 to 100%, respectively (18). Next, GC-TOF-MS was used to analyze exhaled breath from 105 children, comprising 48 children with CF and 57 controls (18). Selection of a distinctive VOC profile consisting of 22 VOCs enabled 100% correct discrimination of children with CF from healthy controls. Within the CF group, 100% correct identification of patients with or without a *P. aeruginosa*-positive culture was possible with only 14 VOCs. The discriminatory compounds were mostly hydrocarbons with 5 to 16 carbon atoms (18). SPME-MS was employed to analyze 72 headspace samples from 72 sputum samples originating from 13 pa-

tients with CF and 59 with non-CF bronchiectasis, of which 32 had positive *P. aeruginosa* cultures, 28 were positive for other pathogens, and the remaining 12 were culture negative (102). It was reported that the VOC 2-nonanone alone could identify *P. aeruginosa* in sputum headspace samples with 72% sensitivity and 88% specificity. A software-assisted sputum library of a set of 17 VOCs which had a sensitivity of detection of *P. aeruginosa* of 62% and a specificity of 100% when used in combination with 2-nonanone was able to detect *P. aeruginosa* in headspace samples with 91% sensitivity and 88% specificity (102).

***Aspergillus fumigatus*.** *Aspergillus fumigatus* is the etiologic agent of invasive aspergillosis, allergic bronchopulmonary aspergillosis, and chronic fungal sinusitis. This fungal pathogen poses a significant threat for immunocompromised individuals, and early diagnosis of colonized or infected individuals remains difficult (103). Identification of infection by detecting a pathogen-specific volatile biomarker(s) in the breath may offer a useful noninvasive diagnostic tool for early detection and treatment, thus reducing mortality in immunocompromised patients.

2-Pentylfuran (2-PF), a volatile compound, has been detected in the headspace of *Aspergillus fumigatus* cultures (104, 105) and has also been identified in small quantities in the breath samples of CF patients positive for *Aspergillus* but not in the breath samples of healthy controls (105). SPME-GC-MS enabled the detection of 2-PF in the exhaled breath of 17 out of 32 patients with a variety of lung disorders who were at risk of colonization or infection with *Aspergillus*, whereas it was undetectable in 14 healthy subjects, in 1 of 10 neutropenic patients, and in 16 of the 32 patients with lung disorders. Of 10 neutropenic patients not thought to be at risk for *Aspergillus* infection, 1 patient showed a 2-PF-positive result. The sensitivity and specificity of the 2-PF breath test compared with repeated positive *Aspergillus* isolations from sputum or bronchial lavage samples were 77% and 78%, respectively (105). Of special interest is the fact that breath samples from 2 patients with invasive aspergillosis, which tested positive for 2-PF, became negative for this marker after treatment, suggesting possible uses for disease diagnosis and monitoring of disease progression (106). Because of the presence of 2-PF in various food products, it has been suggested that the 2-PF breath test should be performed on samples obtained from patients whose mouth has been rinsed 30 min or more following the ingestion of food material (107).

Gastrointestinal Infections

The etiology of gastrointestinal diseases is poorly understood, and the diagnostic approaches are often cumbersome, occasionally invasive, and time-consuming. VOC biomarker-based tests will offer a rapid and noninvasive alternative.

VOCs from the headspaces of stool samples originating from 35 patients suffering from infectious diarrhea and 6 healthy controls were examined by using the SPME-GC-MS technique (108). Characteristic volatile profiles were observed depending upon the causative organisms; e.g., the absence of hydrocarbons and terpenes indicated infection with *Campylobacter*, and the absence of furan species without indoles was indicative of infection with *Clostridium difficile*. Another study analyzed volatiles emitted by fecal samples from patients with ulcerative colitis disease, a disease characterized by inflammation of the colonic mucosa ($n = 18$); infection with *Campylobacter jejuni* ($n = 31$); and *Clostridium difficile* infection ($n = 22$) and from 30 asymptomatic donors by using the SPME-GC-MS technique (13). By using a mass spectral

NIST 05 library, a total of 297 and 292 volatiles could be identified in cohort and longitudinal studies, respectively. Whereas at least 40 VOCs could be detected in all samples irrespective of the underlying clinical disease, distinct VOC marker patterns were linked to specific clinical disease compared to the VOC marker patterns emanating from the feces of healthy donors (13).

Reportedly, fecal samples of patients from Bangladesh with *Vibrio cholerae* infection (cholera) contained significantly lower numbers of VOCs than did samples from healthy donors (109). The presence of *p*-menth-1-en-8-ol has been found to be specific for fecal samples originating from patients with cholera, and therefore, this VOC is considered a candidate biomarker for detection of infection with *Vibrio cholerae* (17, 109).

H. pylori, which is the cause of a common bacterial infection of the stomach, plays an important role in gastric cancer (110). Urea breath tests commonly used as a noninvasive method for detecting *H. pylori* infections are based on assessing the release of $^{13}\text{CO}_2$ following the hydrolysis of the orally administered substrate [^{13}C]urea by the enzyme urease, which is produced by the bacterium in the stomach (111).

Analysis of exhaled-breath samples from patients infected with *H. pylori* ($n = 6$) and uninfected healthy controls ($n = 23$) by using SPME-GC-MS allowed the detection of three endogenous VOCs, namely, isobutane, 2-butanone, and ethyl acetate, in breath samples of *H. pylori*-infected patients and in the headspace of cultured *H. pylori* strains but not in breath samples of uninfected healthy controls (112). These authors argued that either these three endogenous VOCs were produced by *H. pylori* *in vivo* or they represent host metabolites as a result of infection. Canonical analysis of data allowed discrimination between *H. pylori*-infected and healthy subjects based on these three endogenous VOCs (112). In a different study, using PTR-MS analysis of exhaled breath, elevated levels of HCN and hydrogen nitrate were detected in patients with *H. pylori* gastritis compared to healthy controls (93). Furthermore, HCN has also been detected in the headspace of a cultured *H. pylori* reference strain (NCTC11637) (92). As mentioned above, HCN is also known to be emitted by *P. aeruginosa* cultures (85–87) and is also detectable in exhaled-breath samples of *P. aeruginosa*-infected individuals (88, 101).

Urinary Tract Infections

Urinary tract infections (UTIs) are frequently caused by bacterial pathogens such as *Escherichia coli*, *Proteus* sp., enterococci, *Klebsiella* sp., and *Staphylococcus saprophyticus*. A number of urine sampling procedures and analytical methodologies have been used to identify potential volatile markers of specific bacterial species responsible for UTI (12). Urine is known to contain a complex mixture of a large number of VOCs (113), which are anticipated to show significant alterations after infection and may behave as potential disease markers. Therefore, a number of studies have investigated the use of secondary metabolites produced by bacterial strains to detect UTI. Analytical procedures such as GC-MS have demonstrated the feasibility of detecting UTI via the identification of bacterial volatile metabolites in urine samples following short incubation periods with appropriate precursor compounds (114–117). While this approach made it possible to identify several characteristic high-abundance volatiles produced by specific pathogens, the procedure lacked accuracy and suitability for routine clinical use (12).

Commercially available e-noses have also been used to identify

UTI using urine samples following volatile generation *in vitro* (118). The commercially available e-nose (Bloodhound BH-114) based on 14 conducting polymer (CP) gas sensors identified infections in the urinary tract of 45 suspected cases (34). Another commercial headspace analysis device consisting of an array of conducting sensors (Osmetech microbial analyzer) detected bacteriuria in 534 clinical urine samples with 72% sensitivity and 90% specificity when bacteriuria was defined as $>10^4$ CFU/ml and with sensitivity and specificity values of 83% and 88%, respectively, when bacteriuria was defined as $>10^5$ CFU/ml (119). The sensitive detection of pathogen-specific volatile profiles directly in a urine sample (without preincubation or without isolation of the pathogen) is needed for the efficient routine diagnosis of UTI. In this connection, it is worth noting that the use of SPME combined with two-dimensional GC-TOF-MS has enabled the identification of 28 more VOCs associated with the presence of *P. aeruginosa* than those identified by GC-MS (120). Bacterial volatile profiling of clinical urine samples with sensitive methodologies may prove useful for diagnosing UTI *in situ*.

Other Infections

In addition to the studies described above, there are a few brief reports in the literature which have examined the potential of VOC-based testing for direct detection of certain other clinical conditions resulting from infections caused by different pathogens.

A number of bacterial pathogens are responsible for the infection of the sinus (sinusitis), including, among others, *Staphylococcus aureus*, *P. aeruginosa*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. It has been shown that pure cultures of these individual bacterial species emit distinct VOCs in their headspaces (121). E-nose analysis of nasal outbreath samples from patients with chronic sinusitis correctly discriminated diseased from healthy individuals in 80% of the samples (122). In a separate study, the authors reported the use of an e-nose based on GC and surface acoustic wave sensors (Z-nose). Using this technology, these authors identified 6 VOCs (C_6 to C_{14}) of potential relevance in the diagnosis of chronic sinusitis (123). SPME-GC-MS analysis of volatiles from infected sinus mucus samples showed many bacterium-specific VOCs, which were also identifiable in the headspace of *in vitro* cultures of individual species (121). Interestingly, the concentrations and characteristics of pathogen-associated VOCs emitted from infected mucus differed from those detected in the headspace of cultures representing the same bacterial species which caused the infection. A possible explanation for this finding could be that the nutritional environment and resident bacterial flora of the sinuses account for this difference (121).

Pneumonia, which is characterized by inflammation of the lungs, results from infection with several organisms, including bacterial pathogens, with the most common bacterial pathogens involved in nosocomial pneumonia being *S. pneumoniae*, *Staphylococcus aureus*, *P. aeruginosa*, and *Haemophilus influenzae*. Diagnosis is usually based on clinical findings, chest X ray, and isolation and subsequent identification of the pathogen in culture from bronchial secretions. E-nose (Cyranose 320) analysis of exhaled breath has been shown to discriminate between patients with ventilator-associated pneumonia and healthy controls (124). Promising results have also been reported in a prospective study which showed a good correlation between breath analysis and clinical pneumonia scores (125, 126).

Reportedly, leg ulcers caused by beta-hemolytic streptococci can be distinguished from uninfected ulcers on the basis of volatiles detected in contact dressings of the wound (127). An e-nose based on an array of metal-oxide semiconductor sensors was used to monitor bacterial volatiles in the headspaces from swabs and dressings taken from infected wounds and from uninfected swabs. Principal component analysis showed that volatile profiles identified from dressings of infected wounds may be used to discriminate infected from uninfected patients (128). In particular, reportedly, patterns of volatiles released from dressings of chronic wounds can be used to monitor the progress of wound healing (129). A recent study emphasized the usefulness of a suitable sampling procedure regarding wounds and showed that multivariate data analysis of VOC profiles obtained by GC-MS could discriminate infected chronic wounds from healthy skin (130).

Recently, it was reported that an e-nose can be used to distinguish VOCs emitted by urine samples from patients with bacterial vaginosis from those without infection (131, 132). Furthermore, breath samples from individuals with different respiratory diseases analyzed by using multicapillary chromatography coupled to an ion mobile spectrometer revealed the presence of specific peaks that were unique to the fungal pathogen *Candida*, compared to breath of patients with emphysema or general inflammation (133).

A list of reported VOC biomarkers in relation to some infections is provided in Table 3. Table 4 summarizes published studies related to diagnostic performance of VOC analyses for certain diseases.

CONCLUSIONS AND PERSPECTIVES

The rationale for measuring VOC signals/profiles in complex clinical matrices is to identify unique fingerprints/smell prints which are associated with certain diseases and can provide clues for early disease detection and diagnosis. In this review, we summarize studies which have used emerging technologies for VOC analysis in clinical samples for assessing their diagnostic potential with respect to certain infectious diseases. In contrast to numerous studies reviewed elsewhere, which have described the use of conventional GC-MS and VOC sensing devices for defining the volatile patterns/smell prints of *in vitro*-cultured organisms for identification and classification purposes, relatively few reports are available concerning the applicability of the VOC profiling approach directly on clinical samples with the aim of detecting infectious diseases. The major current limitations of the VOC profiling approach are the extremely high costs of laboratory instrumentation depending on appropriate VOC-detecting technologies and the lack of standardized sample collection and pre-concentration procedures (devices), which are essential for effective clinical implementation.

Together, the studies included in this review have identified VOC biomarkers/profiles for certain infections which allowed discrimination between diseased and healthy individuals. It may be mentioned that the studies reviewed are diverse with respect to the use of different procedures for collection and anatomical collection sites and have used a wide array of detection devices, and as such, it is not possible to draw any conclusions regarding the efficacy of the analytics used. It is also important to note that most of the reported study designs have compared VOCs from subjects with a particular disease condition with those from healthy controls. Only a few investigations have assessed breath VOCs from

TABLE 3 Potential VOCs reported in the literature as indicators/discriminators of some infections

Pathogen and disease	Source	Analysis method	VOC biomarker (no. of VOCs) ^a	Reference
<i>Mycobacterium tuberculosis</i>				
TB	Breath	GC-MS	Methyl nicotinate	70
TB	Breath	GC-MS	Profile (14)	71
TB (active)	Breath	GC-MS	Profile (10)	72
TB	Breath	GC-MS	Profile (8)	73
TB	Breath	GC-TOF-MS	Profile (7)	74
TB	Urine	GC-MS	Profile (5)	78
<i>Pseudomonas aeruginosa</i>				
Lung infection (CF patients)	Breath	SIFT-MS	Hydrogen cyanide	88
	Breath	SIFT-MS	Methylthiocyanate	94
	Breath	GC-MS	2-Aminoacetophenone	99
	Breath	IMS	Profile (21)	101
	Breath	GC-TOF-MS	Profile (22)	18
	Sputum	SPME-GC-MS	2-Nonanone and 2,4-dimethyl-1-heptene	102
<i>Aspergillus fumigatus</i>				
Lung infection	Breath	GC-MS	2-Pentylfuran	105
<i>Vibrio cholerae</i>				
Cholera	Feces	GC-MS	<i>p</i> -Menth-1-en-8-ol	108
<i>Helicobacter pylori</i> infections				
	Breath	GC-MS	Isobutane/2-butanone and ethyl acetate	112
	Breath	PTR-MS	Hydrogen cyanide/hydrogen nitrate	93

^a Numbers in parentheses indicate the number of discriminatory VOCs in the profile.

healthy participants and compared these with those from patients in different stages of a chronic disease (such as a comparison of patients with active TB and controls with latent TB) or in the presence of concomitant dissimilar infections (such as TB coinfection with human immunodeficiency virus).

The accumulated evidence shows that VOC signals measured in exhaled breath as well as in headspaces of feces or urine samples can be used for assessing their association with certain infections. A study using a domestic-animal model of chronic intestinal TB (*M. avium* subsp. *paratuberculosis*) reported that VOC discriminatory attributes were stronger in the breath of animals than in feces (79). However, comparative assessments of disease-detecting efficacy of VOC biomarkers in human exhaled breath, urine,

or feces from the same individual are lacking, and as such, no conclusions are yet possible regarding the disease-detecting strength of VOCs from one source over another. Because breath samples are easy to collect, particularly from children and elderly patients, and instruments for breath analysis are commercially available, this appears to be a promising source of VOC detection in the context of infectious disease diagnosis.

Basically, there are two different approaches used for selecting VOC biomarkers for detecting infections. The first approach, which relates to the use of pathogen-specific biomarkers (single or multiple) for detecting an infection under consideration, assumes that the candidate biomarker(s) is constantly present in the patients (24). It needs to be emphasized that except for a few unique

TABLE 4 Summary of published studies regarding the diagnostic performance of VOC profiles in detecting infectious diseases

Pathogen and disease	Sample source	VOC biomarker (no. of VOCs) ^a	No. of patients ^d			Sensitivity/ specificity (%)	Reference
			DPG	ODG	HCG		
<i>Mycobacterium tuberculosis</i>							
TB	Breath	Profile (14)	23 ^b	19	59	96/79	71
Active TB	Breath	Profile (10)	226 ^c			84/65	72
Active TB	Breath	Profile (8)	130		121	72/72	73
TB	Breath	Profile (7)	50 ^b		50	72/86	74
			21 ^b	50		62/84	
<i>Pseudomonas aeruginosa</i>							
Lung infection in patients with CF	Breath	Profile (21)	24		29	89/77	101
	Breath	Profile (14)	48		57	100/100	18
CF and non-CF bronchiectasis	Sputum	Profile (17)	32	28	12	91/88	102

^a Numbers in parentheses indicate the number of discriminatory VOCs.

^b TB culture positive.

^c Suspected TB.

^d DPG, disease patient group; ODG, other diseases group; HCG, healthy control group.

volatiles, the appearance and/or aberrations in the concentrations of certain VOCs may constantly change because of ongoing endogenous metabolic processes and therefore may not suffice for diagnosis of a disease but may have a role to play in monitoring of the disease status. In addition, the specificity of a test based on a single biomarker is limited by other pathogens producing identical volatile markers. The second approach, which is based upon a combination of pathogen-specific biomarkers and those generated *in vivo* during reciprocal host-pathogen interactions, has been successfully utilized for TB detection (71–73).

In the clinical studies described above, results of potential clinical value have been reported by Michael Phillips and coworkers, who evaluated the performance of TB-related breath VOC profiles on a large number of TB patients by using a convenient POC system (72, 73). However, monitoring of changes/alterations in these biomarkers during treatment and their prognostic significance remain to be evaluated.

It needs to be emphasized that most of the other studies reviewed here have not yet validated the data on a sufficiently large number of subjects in clinical settings. According to ATS/ERS recommendations for the clinical screening of a disease, a breath test should be at least 90% sensitive and 95% specific (134). Furthermore, because VOCs are ubiquitous in the environment and food materials, and there is a risk of contamination from volatiles originating from anaerobes (135), it is necessary to take into consideration possible confounding factors such as life-style factors and geographical locations, which can lead to erroneous results (100, 107).

It is foreseeable that the identification of specific disease-associated VOC marker profiles for a particular disease will ultimately pave the way for the use of this novel technology in routine clinical practice. A number of online VOC detection bench-type instruments based on different technologies (e.g., PTR-MS, SIFT-MS, and IMS) are suitable for POC testing in clinical settings. It may also be possible to design disease-specific sensor arrays targeting unique disease-related VOCs. POC devices (e-noses) based on such specific sensor arrays may allow accurate sensing of disease-related volatiles and could therefore be a valuable tool in daily clinical practice in the near future. As our knowledge increases regarding the microbiomes associated with health and disease as well as the metabolic origins of secreted volatile metabolites from affected organs, bodily surfaces, and secretions, further levels of information and sophistication will be incorporated into devices enabling the detection and analysis of VOCs.

REFERENCES

- Risby TH. 2002. Volatile organic compounds as markers in normal and diseased states, p 113. In *Proceedings of the NATO Advanced Study Institute on Disease Markers in Exhaled Breath*, 22 June to 1 July 2001, Limin Hersonissou, Crete, Greece.
- Shirasu M, Touhara K. 2011. The scent of disease: volatile organic compounds of the human body related to disease and disorders. *J. Biochem.* 150:257–266.
- Dummer J, Storer Swanney M, McEwan M, Scott-Thomsd A, Bhandari S, Chambers S, Dweik R, Epton M. 2011. Analysis of biogenic volatile organic compounds in health and disease. *Trends Anal. Chem.* 30:960–967.
- Cicoletta A. 2008. Volatile organic compounds (VOC): definition, classification and properties. *Rev. Mal. Respir.* 25:155–163.
- Phillips M. 1992. Breath tests in medicine. *Sci. Am.* 7:74–79.
- Miekisch W, Schubert JK, Gabriele FE, Noelge-Schomburg GFE. 2004. Diagnostic potential of breath analysis—focus on volatile organic compounds. *Clin. Chim. Acta* 347:25–39.
- Amann A, Smith D (ed). 2005. *Breath analysis for clinical diagnosis and therapeutic monitoring*. World Scientific, Singapore, Republic of Singapore.
- Cao W, Duan Y. 2006. Breath analysis: potential for clinical diagnosis and exposure assessment. *Clin. Chem.* 52:800–811.
- Smith D, Spanel P. 2007. The challenge of breath analysis for clinical diagnosis and therapeutic monitoring. *Analyst* 132:390–396.
- Boots AW, van Berkel JBN, Dallinga JW, Smolinska A, Wouters MFM, van Schooten FJ. 2012. The versatile use of exhaled volatile organic compounds in health and disease. *J. Breath Res.* 6:027108. doi:10.1088/1752-7155/6/2/027108.
- Buszewski B, Keszy M, Ligor T, Amann T. 2007. Human exhaled air analytics: biomarker of diseases. *Biomed. Chromatogr.* 21:553–566.
- Guernion N, Ratcliffe M, Spencer-Phillips PT, Howe RA. 2001. Identifying bacteria in human urine: current practice and the potential for rapid, near-patient diagnosis by sensing volatile organic compounds. *Clin. Chem. Lab. Med.* 39:893–906.
- Garner CE, Smith S, Costello B, White P, Spencer R, Prober CSJ, Ratcliffe M. 2007. Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *FASEB J.* 1675–1688.
- Phillips M, Cataneo RN, Cheema T, Greenberg J. 2004. Increased breath biomarkers of oxidative stress in diabetes mellitus. *Clin. Chim. Acta* 344:189–194.
- Galassetti PR, Novak B, Nemet D, Rose-Gottron C, Cooper DM, Meinardi S, Neocomb R, Zaldivar F, Blake DR. 2005. Breath ethanol and acetone as indicators of serum glucose levels: an initial report. *Diabetes Technol. Ther.* 7:115–123.
- Novak BJ, Blake DR, Meinardi S, Rowland FS, Pontello A, Cooper DM, Galassetti PR. 2007. Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 104:15613–15618.
- Probert CSJ, Ahmed I, Khalid T, Johnson E, Smith S, Ratcliffe N. 2009. Volatile organic compounds as diagnostic biomarkers in gastrointestinal and liver diseases. *J. Gastrointest. Liver Dis.* 18:337–343.
- Robroeks CM, van Berkel JBN, Dallinga JW, Jobsis Q, Zimmermann LJI, Hendriks HJE, Wouters MFM, van der Grinten CPM, van de Kant KDG, van Schooten FJ, Dompeling E. 2010. Metabolomics of volatile organic compounds in cystic fibrosis patients and controls. *Pediatr. Res.* 68:75–80.
- Phillips M, Cataneo RN, Dittkoff BN, Fisher P, Greenberg J, Gunawardena R, Kwon CS, Rahbari-Oskoui F, Wong C. 2003. Volatile biomarkers of breast cancer in the breath. *Breast J.* 9:184–191.
- Mazzone PJ. 2008. Analysis of volatile organic compounds in the exhaled breath for the diagnosis of lung cancer. *J. Thorac. Oncol.* 3:774–780.
- Horvath I, Lazar Z, Gyulai N, Kollai M, Losonczy G. 2009. Exhaled biomarkers in lung cancer. *Eur. Respir. J.* 34:261–275.
- Hakim M, Broza YY, Barash O, Peled N, Phillips M, Amann A, Haick H. 2012. Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem. Rev.* 112:5949–5966.
- Phillips M, Cataneo RN, Condos R, Ring Erikson GA, Greenberg J, La Bombardi V, Munawar MI, Tietje O. 2007. Volatile biomarkers of pulmonary tuberculosis in the breath. *Tuberculosis* 87:44–52.
- Chambers ST, Scott-Thomas A, Epton M. 2012. Developments in novel breath tests for bacterial and fungal pulmonary infection. *Curr. Opin. Pulm. Med.* 18:228–232.
- Van den Velde S, Nevens F, Van Hee P, van Steenberghe D, Quirynen M. 2008. GC-MS analysis of breath odor compounds in liver patients. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 875:344–348.
- Vereb H, Dietrich AM, Alfeeli B, Agah M. 2011. The possibilities will take your breath away: breath analysis for assessing environmental exposure. *Environ. Sci. Technol.* 45:8167–8175.
- Cao W, Duan Y. 2007. Current status of methods and techniques for breath analysis. *Crit. Rev. Anat. Chem.* 37:3–13.
- Herbig J, Müller M, Schallhart S, Titzmann T, Graus M, Hansel A. 2009. On-line breath analysis with PTR-TOF. *J. Breath Res.* 3:027004. doi:10.1088/1752-7155/3/2/027004.
- Zhang Z, Li JA. 2010. The review of advances and new developments in the analysis of biological volatile organic compounds. *Microchem. J.* 95:127–139.
- Wojtas J, Bielecki Z, Stacewicz T, Mikolajczyk J, Nowakowski M. 2012. Ultrasensitive laser spectroscopy for breath analysis. *Opto-Electron. Rev.* 20:26–39.

31. Pavlou AK, Turner APF. 2000. Sniffing out the truth: clinical diagnosis using the electronic nose. *Clin. Lab. Med.* 38:99–112.
32. Turner APF, Magan N. 2004. Electronic noses and disease diagnostics. *Nat. Rev. Microbiol.* 2:161–166.
33. Shnayderman M, Mansfield B, Yip P, Clark HA, Krebs MD, Cohen SJ, Zeskind JE, Ryan ET, Dorkin HL, Callahan MV, Stair TO, Gelfand JA, Gill CJ, Hitt B, Davis CE. 2005. Species-specific bacteria identification using differential mobility spectrometry and bioinformatics pattern recognition. *Anal. Chem.* 77:5930–5937.
34. Schulz S, Dickschat JS. 2007. Bacterial volatiles: the smell of small organisms. *Nat. Prod. Rep.* 24:814–842.
35. Jünger M, Vautz W, Kuhns M, Hofmann L, Ulbricht S, Baumbach JJ, Quintel M, Perl T. 2012. Ion mobility spectrometry for microbial volatile organic compounds: a new identification tool for human pathogenic bacteria. *Appl. Microbiol. Biotechnol.* 93:2603–2614.
36. Steeghs MML, Cristescu SM, Harren FJM. 2007. The suitability of Tedlar bags for breath sampling in medical diagnostic research. *Physiol. Meas.* 28:73–84.
37. Pleil JD, Lindstrom AB. 1995. Measurement of volatile organic compounds in exhaled breath as collected in evacuated electropolished canisters. *J. Chromatogr. B Biomed. Appl.* 665:271–279.
38. Phillips M. 1997. Method for the collection and assay of volatile organic compounds in breath. *Anal. Biochem.* 247:272–278.
39. Grote C, Pawliszyn J. 1997. Solid-phase microextraction for the analysis of human breath. *Anal. Chem.* 69:587–596.
40. Lord H, Yu Y, Segal A, Pawliszyn J. 2002. Breath analysis and monitoring by membrane extraction with sorbent interface. *Anal. Chem.* 74:5650–5657.
41. Effros RM. 2010. Exhaled breath condensate: delusion or dilution? *Chest* 138:471–472.
42. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jöbsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, Wirtz H, ATS/ERS Task Force on Exhaled Breath Condensate. 2005. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur. Respir. J.* 26:523–548.
43. Kneepkens CME, Lepage G, Roy CC. 1994. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic. Biol. Med.* 17:127–160.
44. Baumbach JJ. 2008. Ion mobility spectrometry coupled with multicapillary columns for metabolic profiling of human breath. *J. Breath Res.* 3:034001. doi:10.1088/1752-7155/3/3/034001.
45. Amann A, Poupard G, Telser S, Ledochowski M, Schmidt A, Mechtchriakov S. 2004. Applications of breath gas analysis in medicine. *Int. J. Mass Spectrom.* 239(2–3):227–233.
46. Spanel P, Smith D. 2011. Progress in SIFT-MS: breath analysis and other applications. *Mass Spectrom. Rev.* 30:236–267.
47. Maniewski R, Liebert A, Kacprzak M, Zbiec A. 2004. Selected application of near-infrared optical methods in medical diagnosis. *Opto-Electron. Rev.* 12:255–262.
48. Rock F, Barsan N, Weimar U. 2008. Electronic nose: current status and future trends. *Chem. Rev.* 108:705–725.
49. Wilson AD, Baietto M. 2011. Advances in electronic-nose technologies developed for biomedical applications. *Sensors* 11:1105–1176.
50. Hines EL, Lloblet E, Gardner JW. 1996. Electron noses: review of signal processing techniques. *IEE Proc. Circuits Syst.* 146:297–310.
51. Tisch U, Haick H. 2010. Nanomaterials for cross reactive sensor arrays. *MRS Bull.* 35:797–803.
52. Peng G, Tisch U, Adams O, Hakim M, Shehada N, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, Haick H. 2009. Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nat. Nanotechnol.* 4:669–673.
53. Pearce TC. 1997. Computational parallels between the biological olfactory pathway and its analogue in electronic nose part II: sensor based machine olfaction. *Biosystems* 41:69–90.
54. Staples EJ. 1999. A new electronic nose. *Sensors* 16:33–40.
55. Staples EJ. 2000. The z Nose, a new electronic nose using acoustic technology. *J. Acoust. Soc. Am.* 108:2495.
56. Moser E, McCulloch M. 2010. Canine scent detection of human cancers. A review of methods and accuracy. *J. Vet. Behav.* 5:145–152.
57. Weetjens BJ, Mgoe GF, Machangu RS, Kazwala R, Mfinanga G, Lwilla F, Cox C, Jubitana A, Kanyagha H, Mtandu R, Kahwa A, Mwessongo J, Makingi G, Mfaume S, Van Steenberghe J, Beyene NW, Billet M, Verhagen R. 2009. African pouched rats for the identification of pulmonary tuberculosis in sputum. *Int. J. Tuberc. Lung Dis.* 13:737–743.
58. Stoltzky G, Schneck S. 1976. Volatile organic compounds and microorganisms. *Crit. Rev. Microbiol.* 4:333–382.
59. Lechner M, Fille M, Hausdorfer J, Dierich MP, Rieder J. 2005. Diagnosis of bacteria in vitro by mass spectrometric fingerprinting: a pilot study. *Curr. Microbiol.* 51:267–269.
60. Bruins M, Bos A, Petit PLC, Eadie K, Rog A, Bos R, van Ramshorst GH, van Belkum A. 2009. Device-independent, real-time identification of bacterial pathogens with a metal oxide-based olfactory sensor. *Eur. J. Clin. Microbiol. Infect. Dis.* 28:775–780.
61. Lawn SD, Zumla A. 2011. Tuberculosis. *Lancet* 378:57–72.
62. Wallis RS, Paiu M, Menzies D, Doherty TM, Walzl G, Perkins MD, Zumla A. 2010. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet* 375:1920–1937.
63. Nahid P, Kim PS, Evans CA, Alland D, Barer M, Diefenbach J, Ellner J, Hafner R, Hamilton CD, Iademarco MF, Ireton G, Kimmerling ME, Lienhardt C, MacKenzie WR, Murray M, Perkins MD, Posey JE, Roberts T, Sizemore C, Stevens WS, Via L, Williams SD, Yew WW, Swindells S. 2012. Clinical research and development of tuberculosis diagnostics: moving from silos to synergy. *J. Infect. Dis.* 205(Suppl 2): S159–S168. doi:10.1093/infdis/jis194.
64. McNerney R, Daley P. 2011. Towards a point-of-care test for active tuberculosis: obstacles and opportunities. *Nat. Rev. Microbiol.* 9:204–213.
65. Omelianski VL. 1923. Aroma-producing microorganisms. *J. Bacteriol.* 8:393–419.
66. Pavlou AK, Magan N, Jones J, Brown J, Klaster P, Turner A. 2004. Detection of *Mycobacterium tuberculosis* (TB) in vitro and in situ using electronic nose detection in combination with a neural network system. *Biosens. Bioelectron.* 20:538–544.
67. Fend R, Kolk AHJ, Bessant C, Buijts P, Klaster PR, Woodman AC. 2006. Prospects for clinical application of electronic-nose technology to early detection of *Mycobacterium tuberculosis* in culture and sputum. *J. Clin. Microbiol.* 44:2039–2045.
68. Kolk A, Hoelscher M, Maboko L, Jung J, Kuijper S, Cauchiu M, Bessant C, van Beers S, Dutta R, Gibson T, Reither K. 2010. Electron nose technology using sputum samples in diagnosis of patients with tuberculosis. *J. Clin. Microbiol.* 48:4235–4238.
69. Syhre M, Chambers ST. 2008. The scent of *Mycobacterium tuberculosis*. *Tuberculosis (Edinb.)* 88:317–323.
70. Syhre M, Manning L, Phuanukoonnon S, Harino P, Chambers ST. 2009. The scent of *Mycobacterium tuberculosis*—part II breath. *Tuberculosis (Edinb.)* 89:263–266.
71. Phillips M, Cantaneo RN, Condos R, Gerald A, Erickson R, Greenberg J, La Bombardi V, Munawar MI, Tietje O. 2007. Volatile biomarkers of pulmonary tuberculosis in the breath. *Tuberculosis (Edinb.)* 87:44–52.
72. Phillips M, Basa-Dalay V, Bothamley G, Cataneo RN, Lam PK, Natividad MPR, Schmitt P, Wai J. 2010. Breath biomarkers of active pulmonary tuberculosis. *Tuberculosis (Edinb.)* 90:145–151.
73. Phillips M, Basa-Dalay V, Blais J, Bothamley G, Chaturvedi A, Modi KD, Pandya M, Natividad MPR, Patel U, Ramraje NN, Schmitt P, Udawadia ZF. 2012. Point-of-care breath test for biomarkers of active tuberculosis. *Tuberculosis (Edinb.)* 92:314–332.
74. Kolk AHJ, Van Berkel JBN, Claassens MM, Walters E, Kuijper S, Dallinga JW, van Schooten FJ. 2012. Breath analysis as a potential diagnostic tool for tuberculosis. *Int. J. Tuberc. Lung Dis.* 16:777–782.
75. Risby TH, Sehnert SS. 1999. Clinical application of breath biomarkers of oxidative stress status. *Free Radic. Biol. Med.* 27:1182–1192.
76. Aghdassi E, Allard JP. 2000. Breath alkanes as a marker of oxidative stress in different clinical conditions. *Free Radic. Biol. Med.* 28:880–886.
77. Quinn MT, Ammons MCB, Deleo FR. 2006. The expanding role of NADPH oxidases in health and disease: no longer just agents of death and destruction. *Clin. Sci.* 111:1–20.
78. Banday KM, Pasikanti KK, Chan ECY, Singla R, Rao KV, Chauhan VS, Nanda RK. 2011. Use of urine volatile organic compounds to discriminate tuberculosis patients from healthy subjects. *Anal. Chem.* 83: 5526–5534.
79. Purkhart R, Köhler H, Liebler-Tenorio E, Meyer M, Becher G, Kikowitz A, Reinhold P. 2011. Chronic intestinal *Mycobacterium* infection:

- discrimination via VOC analysis in exhaled breath and headspace of feces using differential ion mobility. *J. Breath Res.* 5:027103. doi:[10.1088/1752-7155/5/2/027103](https://doi.org/10.1088/1752-7155/5/2/027103).
80. Peled N, Ionescu R, Nol P, Barash O, McCollum M, VerCauteren K, Koslow M, Stahl R, Rhyan J, Haick H. 2012. Detection of volatile organic compounds in cattle naturally infected with *Mycobacterium bovis*. *Sens. Actuators B Chem.* 171-172:588–594.
  81. Knobloch H, Koehler H, Commander N, Reinhold P, Turner C, Chambers M. 2009. Volatile organic compound (VOC) analysis for disease detection: proof of principle for field studies detecting paratuberculosis and brucellosis, p 195–197. In Pardo M, Sberveglieri G (ed), *CP1137, Olfaction and Electronic Nose: Proceedings of the 13th International Symposium*. American Institute of Physics, College Park, MD.
  82. Fend R, Goddes R, Lesellier S, Vordermeier HM, Corner LAL, Gormley E, Costello E, Hewinson RG, Marlin DJ, Woodman AC, Chambers MA. 2005. Use of an electronic nose to diagnose *Mycobacterium bovis* infection in badgers and cattle. *J. Clin. Microbiol.* 43:1745–1751.
  83. Lyczak JB, Cannon CL, Pier GB. 2000. Establishment of *Pseudomonas* infection: lessons from a versatile opportunist. *Microbes Infect.* 2:1051–1060.
  84. Gibson RL, Burns JL, Ramsey BW. 2003. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 168:918–951.
  85. Castric PA. 1983. The influence of oxygen on the *Pseudomonas aeruginosa* hydrogen cyanide synthase. *Curr. Microbiol.* 29:19–23.
  86. Carterson AJ, Morici LA, Jackson DW, Frisk A, Lizewski SE, Jupiter R, Simpson K, Kunz DA, Davis SH, Schurr JR, Hassett DJ, Schurr MJ. 2004. The transcriptional regulator AlgR controls cyanide production in *Pseudomonas aeruginosa*. *J. Bacteriol.* 186:6837–6844.
  87. Carroll W, Lenney W, Wang T, Spanel P, Alcock A, Smith D. 2005. Detection of volatile compounds by *Pseudomonas aeruginosa* using selected ion flow tube mass spectrometry. *Pediatr. Pulmonol.* 39:452–456.
  88. Enderby B, Smith D, Carroll W, Lenney W. 2009. Hydrogen cyanide as a biomarker for *Pseudomonas aeruginosa* in the breath of children with cystic fibrosis. *Pediatr. Pulmonol.* 44:142–147.
  89. Wang T, Pysanenko A, Dryahina K, Spanel 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. doi:[10.1088/1752-7155/2/3/037013](https://doi.org/10.1088/1752-7155/2/3/037013).
  90. Stamyr K, Vaittinen O, Jaakola J, Guss J, Metsala M, Johanson G, Halonen L. 2009. Background levels of hydrogen cyanide in human breath measured by infrared cavity ring down spectroscopy. *Biomarkers* 14:285–291.
  91. Schmidt FM, Metsälä M, Vaittinen O, Halonen L. 2011. Background levels and diurnal variations of hydrogen cyanide in breath and emitted from skin. *J. Breath Res.* 5:046004. doi:[10.1088/1752-7155/5/4/046004](https://doi.org/10.1088/1752-7155/5/4/046004).
  92. Lechner M, Tilg H, Rieder J. 2006. Analysis of volatile compounds emitted by the *Helicobacter pylori* reference strain NCTC 11637 in vitro. *Helicobacter* 11:66. doi:[10.1111/j.0083-8703.2006.00372.x](https://doi.org/10.1111/j.0083-8703.2006.00372.x).
  93. Lechner M, Karlseder A, Niederseer D, Lirk P, Neher A, Rieder J. 2005. *H. pylori* infection increases levels of exhaled nitrate. *Helicobacter* 10:385–390.
  94. Shestivska V, Nemec A, Drevinek P, Sovova K, Dryahina K, Spanel P. 2011. Quantification of methyl thiocyanate in the headspace of *Pseudomonas aeruginosa* cultures and in the breath of cystic fibrosis patients by selected ion flow tube mass spectrometry. *Rapid Commun. Mass Spectrom.* 25:2459–2467.
  95. Mann S. 1966. Über den Geruchsstoff von *Pseudomonas aeruginosa*. *Arch. Mikrobiol.* 54:184–190.
  96. Habs H, Mann S. 1967. Die Bildung von Ortho-aminoacetophenone durch apocyaninogene Stämme von *Pseudomonas aeruginosa*. *Zentralbl. Bakteriolog. Orig.* 203:473–477.
  97. Cox CD, Parker J. 1979. Use of 2-aminoacetophenone production in identification of *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* 9:479–484.
  98. Labows JN, McGinley KJ, Webster GF, Leyden JH. 1980. Headspace analysis of volatile metabolites of *Pseudomonas aeruginosa* and related species by gas chromatography-mass spectrometry. *J. Clin. Microbiol.* 12:521–526.
  99. Scott-Thomas A, Syhre M, Pattemore PK, Epton MJ, Laing R, Pearson JP, Chambers ST. 2010. 2-Aminoacetophenone as a potential breath biomarker for *Pseudomonas aeruginosa* in the cystic fibrosis lung. *BMC Pulm. Med.* 10:56. doi:[10.1186/1471-2466-10-56](https://doi.org/10.1186/1471-2466-10-56).
  100. Scott-Thomas A, Pearson J, Chambers S. 2011. Potential sources of 2-aminoacetophenone to confound the *Pseudomonas aeruginosa* breath test, including analysis of a food challenge study. *J. Breath Res.* 5:046002. doi:[10.1088/1752-7155/5/4/046002](https://doi.org/10.1088/1752-7155/5/4/046002).
  101. Rabis T, Sommerwerck U, Anhehn O, Darwiche K, Freitag L, Teschler H, Bödeker B, Madulla S, Baumbach JI. 2011. Detection of infectious agents in the airways by ion mobility spectrometry of exhaled breath. *Int. J. Ion Mobil. Spectrom.* 14:187–195.
  102. Savelev SU, Perry JD, Bourke SJ, Jary H, Taylor R, Fisher AJ, Corris PA, Petrie M, De Soya A. 2011. Volatile biomarkers of *Pseudomonas aeruginosa* in cystic fibrosis and noncystic fibrosis bronchiectasis. *Lett. Appl. Microbiol.* 52:610–613.
  103. Lin SJ, Scharanz J, Teutsch SM. 2001. Aspergillosis case fatality rate: systematic review of the literature. *Clin. Infect. Dis.* 32:358–366.
  104. Syhre M, Scotter JM, Chambers ST. 2008. Investigation into the production of 2-pentylfuran by *Aspergillus fumigatus* and other diagnostics: progress toward a breath test for invasive *Aspergillus fumigatus*. *Med. Mycol.* 46:209–215.
  105. Chambers S, Syhre M, Murdoch D, McCartin F, Epton MJ. 2009. Detection of 2-pentylfuran in the breath of patients with *Aspergillus fumigatus*. *Med. Mycol.* 47:468–476.
  106. Chambers ST, Bhandari S, Scott-Thomas A, Syhre M. 2011. Novel diagnostics: progress toward a breath test for invasive *Aspergillus fumigatus*. *Med. Microbiol.* 49(Suppl 1):S54–S61. doi:[10.3109/13693786.2010.508187](https://doi.org/10.3109/13693786.2010.508187).
  107. Bhandari S, Chambers S, Pearson J, Syhre M, Epton M, Scott-Thomas A. 2011. Determining the limits and confounders for the 2-pentylfuran breath test by gas chromatography/mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 879:2815–2820.
  108. Probert CSJ, Jones PRH, Ratcliffe NM. 2004. A novel method for rapidly diagnosing the causes of diarrhea. *Gut* 53:58–61.
  109. Garner CE, Smith S, Bardhan PK, Ratcliffe NM, Probert CS. 2009. A pilot study of faecal volatile organic compounds in faeces from cholera patients in Bangladesh to determine their utility in disease diagnosis. *Trans. R. Soc. Trop. Med. Hyg.* 103:1171–1173.
  110. Pakodi F, Abdel-Salam OME, Debrececi A, Mozsik G. 2000. *Helicobacter pylori*. One bacterium and a broad spectrum of human disease. *J. Physiol. Paris* 94:139–152.
  111. Gisbert JP, Pajares JM. 2004. ¹³C-urea breath test in the diagnosis of *Helicobacter pylori* infection—a critical review. *Aliment. Pharmacol. Ther.* 20:1001–1017.
  112. Ulanowska A, Kowalowski T, Hryniewicz K, Jackowski M, Buszewski B. 2011. Determination of volatile organic compounds in human breath for *Helicobacter pylori* detection by SPME-GC/MS. *Biomed. Chromatogr.* 25:391–397.
  113. Pauling L, Robinson AB, Teranishi R, Cary P. 1971. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc. Natl. Acad. Sci. U. S. A.* 68:2374–2376.
  114. Hayward NJ, Jeavons TH. 1977. Assessment of technique for rapid detection of *Escherichia coli* and *Proteus* species in urine by head-space gas-liquid chromatography. *J. Clin. Microbiol.* 6:202–208.
  115. Coloe PJ. 1978. Headspace gas-liquid chromatography for rapid detection of *Escherichia coli* and *Proteus mirabilis* in urine. *J. Clin. Pathol.* 31:361–364.
  116. Hayward NJ. 1983. Headspace gas-liquid chromatography for the rapid laboratory diagnosis of urinary tract infections caused by enterobacteria. *J. Chromatogr.* 274:27–35.
  117. Davies T, Hayward NJ. 1984. Volatile products from acetylcholine as markers in the rapid urine test using headspace gas-liquid chromatography. *J. Chromatogr.* 307:11–21.
  118. Pavlou A, Magan N, McNulty C, Jones J, Sharp D, Brown J, Turner AP. 2002. Use of an electronic nose system for diagnosis of urinary tract infections in vivo. *Biosens. Bioelectron.* 17:893–899.
  119. Aathithan S, Plant JC, Chaudry AN, French GL. 2001. Diagnosis of bacteriuria by detection of volatile organic compounds in urine using an automated headspace analyzer with multiple conducting polymer sensors. *J. Clin. Microbiol.* 39:2590–2593.
  120. Bean H, Dimandja D, Jean-Marie D, Hill JE. 2012. Bacterial volatile discovery using solid phase microextraction and comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 901:41–46.
  121. Preti G, Thaler E, Hanson CW, Troy M, Eades J, Gelperin A. 2009. Volatile compounds characteristic of sinus-related bacteria and infected sinus mucus: analysis by solid-phase microextraction and gas chroma-

- tography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 877:2011–2018.
122. Mohamed E, Bruno E, Linder R, Alessandrini M, Di Girolamo A, Poppl SJ, De Lorenzo A. 2003. A novel method for diagnosing chronic rhinosinusitis based on an electronic nose. *An. Otorhinolaringol. Ibero Am.* 30:447–457.
  123. Bruno E, Alessandrini M, Ottaviani F, Delfini A, Di Pierro D, Camillo A, De Lorenzo A. 2008. Can the electronic nose diagnose chronic rhinosinusitis? A new experimental study. *Eur. Arch. Otorhinolaryngol.* 265:425–428.
  124. Hockstein NG, Thaler ER, Torigian D, Miller WT, Deffenderfer O, Hanson CW. 2004. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. *Laryngoscope* 114:1701–1705.
  125. Hanson CW, Thaler ER. 2005. Electronic nose prediction of a clinical pneumonia score: biosensors and microbes. *Anesthesiology* 102:63–68.
  126. Hockstein NG, Thaler ER, Lin Y, Lee DD, Hanson CW. 2005. Correlation of pneumonia score with electronic nose signature: a prospective study. *Ann. Otol. Rhinol. Laryngol.* 114:504–508.
  127. Parry AD, Chadwick PR, Simon D, Oppenheim B, McCollum CN. 1995. Leg ulcer odour detection identifies beta-haemolytic streptococcal infection. *J. Wound Care* 4:404–406.
  128. Byun H, Persaud KC, Pisanelli AM. 2010. Wound-state monitoring for burn patients using E-nose/SPME system. *ETRI J.* 32(3):440–446.
  129. Greenwood JE, Crawley BA, Clark SL, Chadwick PR, Ellison DA, Oppenheim BA, McCollum CN. 1997. Monitoring wound healing by odor. *J. Wound Care* 6:219–221.
  130. Thomas AN, Riazanskaia S, Cheung W, Xu Y, Goodacre R, Thomas CLP, Baguneid MS, Bayat A. 2010. Novel noninvasive identification of biomarkers by analytical profiling of chronic wounds using volatile organic compounds. *Wound Repair Regen.* 18:391–400.
  131. Chandiok S, Crawley BA, Oppenheim BA, Chadwick P, Higgins S, Persaud KC. 1997. Screening for bacterial vaginosis: a novel application of artificial nose technology. *J. Clin. Pathol.* 50:790–791.
  132. Hay P, Tummon A, Ogunfile M, Adebisi A, Adefowora A. 2003. Evaluation of a novel diagnostic test for bacterial vaginosis: the electronic nose. *Int. J. STD AIDS* 14:114–118.
  133. Ruzsanyi V, Baumbach JJ, Sielemann S, Litterst P, Westhoff M, Freitag L. 2005. Detection of human metabolites using multicapillary columns coupled to ion mobility spectrometers. *J. Chromatogr. A* 1084: 145–151.
  134. American Thoracic Society, European Respiratory Society. 2005. ATS/ERS recommendations for standardized procedures for the on-line and offline measurement of exhaled lower respiratory nitric acid and nasal nitric oxide, 2005. *Am. J. Respir. Crit. Care Med.* 171:912–930.
  135. Khalid TY, Saad S, Greenman J, de Lacy Costello B, Probert CSJ, Ratcliffe NM. 2013. Volatiles from anaerobes confounding breath biomarker discovery. *J. Breath Res.* 7:017114. doi:10.1088/1752-7155/7/1/017114.

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