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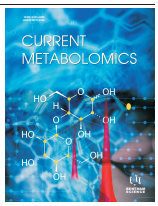
REVIEW ARTICLE

Biomarker Metabolite Signatures Pave the Way for Electronic-nose Applications in Early Clinical Disease Diagnoses



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Abstract: ***Background*:** Analysis of volatile metabolites derived from the human breath or biofluids provides noninvasive means of detecting and monitoring diseases that occur throughout the body. Diseases arise from different mechanisms that cause alterations in normal physiological processes. Mechanisms of disease (pathogenesis) result in the production of unique mixtures of abnormal volatile organic compounds (VOCs), referred to as disease biomarker metabolites when associated with specific diseases. Regardless of where disease biomarkers originate in the body, they are picked up by the circulatory system and eventually expelled out through the lungs. Analysis of complex mixtures of disease biomarkers provides effective diagnostic clues for detecting the presence of specific disease processes occurring in the body.

***Methods*:** Recent progress in the development of electronic-nose (e-nose) applications and technologies for clinical examinations and human disease diagnoses are reviewed.

***Results*:** Metabolomics has been useful in identifying biomarkers and mechanisms of disease, but is often time-consuming and not easily applied to disease diagnosis. E-nose devices are relatively new gas-sensing technologies that are small, simple, portable and particularly useful for noninvasive early disease detection. Some major advantages of using e-noses for disease diagnoses are that they provide quicker, more efficient diagnostic results and cause less stress, anxiety, and no pain to patients.

***Conclusion*:** Recent advancements in the use of e-nose devices to detect complex mixtures of disease biomarkers are providing the great potential for these instruments to facilitate and accelerate point-of-care clinical disease diagnoses.

Keywords: Aroma signature patterns, breathprints, disease biomarkers, electronic aroma detection, e-nose, metabolomics.

1. INTRODUCTION

The use of noninvasive methods for disease detection increasingly is becoming the new trend and goal of many progressive diagnostic laboratories and clinics worldwide. Some keys reasons for this significant shift in diagnostic approaches are that most current conventional chemical analysis technologies, still widely used for clinical diagnostics, are very expensive, time-consuming, highly sophisticated (requiring highly-trained laboratory scientists), and often result in delays in diagnoses and treatments for diseases [1-4]. Other important reasons for shifts toward noninvasive methods include the need to move away from clinical procedures that are painful to patients or cause anxiety that preclude their participation in early prophylactic disease screenings.

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The detection and identification of disease biomarker metabolites recently have become a popular way of studying and diagnosing diseases because this approach allows for a logical chemical connection and explanation for the effects of disease mechanisms in altering normal biochemical cellular process and metabolic pathways in the body. These processes often result in the appearance of abnormal profiles (types and quantities of cellular metabolites) in diseased patients. Use of the metabolomics-approach to early disease detection, involving quantification of the increases or decreases in types and amounts of cellular metabolites, has been quite useful in identifying many potential biomarkers of disease and determining which metabolic pathways are associated with changes in the production of volatile organic metabolites (VOMs) and non-volatile organic metabolites (NVOMs) due to the presence of disease [5]. Similar approaches of early disease diagnoses, using disease fingerprinting, are being developed and applied to the human immune system in order to decode DNA sequences in immune cells that code for specific immunoglobulin proteins (they produce) which are conformational matches to antigenic sites

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on specific types of microbial pathogens that cause certain diseases [6]. The main advantages of early disease detection using these approaches are obtaining precise knowledge of whether infection has already occurred (well in advance of symptom development), the type of invading microbe, the specific strain of microbial pathogen involved, and early indications of the most likely effective treatments if available.

Applications of electronic-nose (e-nose) devices for clinical disease diagnoses continue to be developed with increasing impetus as growing evidence indicates that disease biomarkers are more optimally detected and recognized in aggregation, within complex gaseous mixtures, using e-nose sensor arrays, pattern-recognition algorithms and statistical discrimination procedures [7]. Rather than relying on complex chemical analysis methods to identify individual disease biomarker metabolites in human samples, e-nose instruments may be used to determine disease states by recognizing and distinguishing aggregate patterns of specific mixtures of volatile organic compounds (VOCs) released into the headspace from diagnostic samples. Considerable evidence has shown that single-biomarker approaches to identifying disease are, in most cases, oversimplified and ineffective in providing reliable diagnoses with the exception of certain single-metabolite associated genetic (metabolic) disorders [8]. Similar difficulties in achieving unequivocal diagnoses from metabolomics-type approaches alone, have demonstrated the need for simpler, more effective methods that can be readily used by physicians and clinical laboratory technicians to provide rapid, reliable results for real-time diagnostic point-of-care testing (POCT).

This review provides a thorough comparison of metabolomic versus electronic-nose technologies for their utilities, strengths and weaknesses in providing useful information for clinical diagnoses; and a summary of international research showing current progress in the development of electronic-nose diagnostic methods for clinical practice.

2. EARLY DISEASE DETECTION

Early detection of disease always is of paramount importance in disease diagnostics because it allows for earlier and more effective treatments with greatly improved prognoses for patient recovery [1, 8, 9]. This is particularly true in the case of systemic infections (sepsis) by morbific pathogens where a swift diagnosis can mean the difference between life or death for critically ill patients [10]. In these cases, the identification of specific strains of a microbial pathogen is critical for application of the appropriate antimicrobial therapy to check and kill the invading microbe.

Other instances where rapid diagnoses are essential occur when a serious disease has progressed to advanced stages, requiring an effective treatment to be applied immediately if there is to be any chances for successful patient recovery. In this case, there is no time for a delayed diagnosis through conventional methods involving the slow culturing and identification of microbial pathogens, or complicated and time-consuming chemical-analysis approaches requiring extensive sample preparation and highly-trained laboratory personnel. Instead, a real-time rapid and reliable diagnostic approach is essential for allowing the application of immediate curative treatments [8, 11, 12].

3. DISEASE BIOMARKERS

Analysis of specific combinations of VOCs present in complex gaseous mixtures of exhaled breath and in the headspace of human biofluid samples, including blood (plasma/serum), saliva, semen, sputum, and urine, provides a snapshot view of the overall physiological state of the body at the moment the samples were collected [1, 11]. A smaller subset of key volatiles, the unique biomarker metabolites present in the human breath and biofluids, potentially provide even more information about the healthful or pathological state of the body by virtue of their sources, whether derived from normal physiological pathways or from altered, abnormal pathways associated with disease processes. Consequently, volatile biomarkers can provide very effective clues about specific physiological processes, either benign or adverse, that exist in specific organs or compartments of the body [1, 8, 12]. Metabolomics have been used to study a wide range of diseases and identify numerous biomarker metabolites associated with specific diseases, including both infectious and noninfectious types [2, 5, 10, 11]. This approach has provided greater understanding of normal cellular metabolic pathways and new important clues of the mechanisms by which normal physiological pathways in healthy individuals are affected by disease processes to generate aberrant volatile biomarkers of disease in ill patients.

Metabolic fingerprinting of many major diseases through the identification of key biomarker metabolites, produced as a consequence of disease-process effects on cellular metabolic pathways, has generated a wealth of information to characterize the chemical changes associated with various types of infectious diseases. Most microbial pathogens, as causal agents of disease, depend on the host's genetic machinery and metabolic pathways in order to replicate and multiply by utilizing host metabolites (from cellular pathways) as sources of energy and building materials for both anabolic and catabolic activities. Some examples of the many types of VOMs, produced by disease processes, which have been identified as significant biomarkers for the detection of different categories of specific infectious diseases are summarized in Table **1**. The types and chemical classes of important volatile disease biomarkers, identified through metabolomics, have provided significant insights into the specific metabolic pathways affected by the unique disease processes caused by different categories of microbial pathogens. Differences in the types of VOMs produced by different microbial types indicate differences in the mechanisms of disease used to exploit cellular processes. Viruses usually take over the genetic-replication processes of host cells for virion replication, whereas pathogenic bacteria, fungi, and protozoa produce enzymes, toxins, polysaccharides, and other pathogenic determinants to initiate disease processes that target and affect different types of cellular pathways.

Some of the main metabolic pathways affected by microbial pathogens include amino acid, fatty acid, lipid, nucleoside, glucose, phospholipid, steroid, and hormone metabolism as well as glycolysis and tricarboxylic acid (TCA) cycles [5]. Alterations in these metabolic cycles, caused by

Table 1. Volatile organic metabolites (VOMs) identified as significant biomarkers for detection of specific infectious diseases.

Disease Type	Disease Name	Pathogen	N=	Method	Model	Sample	VOMs	Most Significant Biomarkers (Chemical Class Abbrev.)	Refs.
Bacterial	Gastritis, ulcer	*H. pylori*	20	H-NMR	Gerbils	Urine	8	*cis*-aconitate (tca) Indoxyl sulfate (aad) Proline (aa) Hippurate (bcad)	[13]
	Tuberculosis	*M. tuberculosis*	95	GC-MS	Human	Sputum	22	D-glucosamine (as) N-acetylglucosamine (as) D-glucupyranose (ms) D-glucupyranoside (gsd) 2-deoxy-D-erythro-pentitol (sa) D-galactose-6-deoxy (ms)	[14]
		M. tuberculosis	136	UPLC-MS	Human	Serum	20	Inosine (ns) Hypoxanthine (pud) Glycylalanine (aad) 5-oxoproline (aad)	[15]
		M. tuberculosis	20	GC-MS	Human	Serum	9	5-oxoproline (aad)	[16]
	Salmonellosis	*S. typhimurium*	20	GC-MS	Mice	Gut	5	Lactose (ds) Melibiose (ds) Raffinose (ts) Fucose (ms) Galactinol (sa)	[17]
	Staphylococcosis	*S. aureus*	30	H-NMR	Mice	Urine	4	1-methylnicotinamide (vba) 3-methyl-2-oxyvalerate (cad) 2-oxoisocaproate (cad) n-isovaleroylglycine (aad)	[18]
		S. aureus	17	H-NMR	Mice	Serum	5	Acetone (ket) 3-hydroxybutyrate (bhca) 2-hydroxybutyrate (bhca) Creatine (cad) Isobutyrate (ca)	[19]
	Streptococcosis	*S. pneumoniae*	30	H-NMR	Mice	Urine	5	Ethanolamine (pad) Fucose (ms) Creatine (cad) Taurine (aad)	[18]
		S. pneumoniae	17	H-NMR	Mice	Serum	6	Hippurate (bcad) Glucose (ms) Pyruvate (ca) 2-oxoglutarate (dca) Citrate (tca) Fumarate (dca)	[19]

(Table 1) Contd...

Disease Type	Disease Name	Pathogen	N=	Method	Model	Sample	VOMs	Most Significant Biomarkers (Chemical Class Abbrev.)	Refs.
		S. pneumo-niae	86	H-NMR	Human	Urine	27	Carnitine (aad) Acetylcarnitine (aad) myo-Inositol (sa) 3-hydroxybutyrate (bhca) Taurine (aad)	[20]
Fungal	Aspergillosis	*A. fumigatus*	46	GC-MS	Human	Breath	1	2-pentylfuran (fd)	[21]
Protozoa	Cerebral malaria	*P. berghei*	86	H-NMR, P-MRS	Mice	Brain	5	Glutamine (aa)	[22]
		P. berghei	4	H-NMR	Mice	Plasma	5	Lactate (ca) Pyruvate (ca)	[23]
		P. berghei	4	H-NMR	Mice	Urine	4	Pipecolic acid (had) Phenylacetylglycine (aad) Dimethylamine (sda)	[23]
	Malaria	*P. falcipa-rum*	3	LC-MS	Cell	Erythro-cyte	9	α-ketoglutarate (cad) 5-methylthioadenosine (ns)	[24]
		P. vivax	54	H-NMR	Human	Urine	2	Pipecolic acid (had) Ornithine (aa)	[25]
	Trypanosomiasis	*T. brucei*	25	H-NMR	Mice	Urine	7	4-hydroxyphenylacetate (bcad) 4-hydroxyphenylpyruvate (bcad) Phenylpyruvate (bcad)	[26]
		T. brucei	24	H-NMR	Mice	Urine	10	3-carboxy-2-methyl-3-oxo-propanamine (pad) Tryptophan (aa) 3-methyl-2-oxovalerate (cad) D-3-hydroxybutyrate (bhca) 4-hydroxyphenylacetate (bcad)	[27]
Schistosome	Schistosomiasis	*S. japoni-cum*	60	H-NMR	Human	Urine, plasma	9	3-ureidopropionate (aad)	[28]
		S. mansoni	52	UPLC-MS	Human	Urine	1	Phenylacetylglycine (aad)	[29]
		S. mansoni	20	CE	Human	Urine	20	Phenylacetylglycine (aad) 2-hydroxyphenylacetate (aad) 4-hydroxyphenylacetate (aad) Phenylalanine (aad)	[30]
		S. mansoni	20	H-NMR	Human	Urine	22	p-cresol glucuronide (bcd) Phenylacetylglycine (aad) Hippurate (bcad) 2-oxoadipate (dca)	[31]
		S. mansoni	20	H-NMR, CPMG	Human	Plasma	2	D-3-hydroxybutyrate (bhca) Glycerophosphorylcholine (pcd)	[31]
		S. mansoni	20	H-NMR	Human	Fecal	1	5-aminovalerate (cad)	[31]

(Table 1) Contd…

Disease Type	Disease Name	Pathogen	N=	Method	Model	Sample	VOMs	Most Significant Biomarkers (Chemical Class Abbrev.)	Refs.
Viral	Hepatitis B	CHBV	28	H-NMR	Human	Serum	6	Glucose (ms) Alanine (aa) Valine (aa) Glutamine (aa)	[32]
		CHBV	24	UPLC-MS	Human	Urine	4	Biotin sulfone (vbd) 5-oxy-heneicosanoic acid (fad) Glucosaminide (asd) 2-methylhippuric acid (bcad)	[33]
	Hepatitis C	HCV	14	UPLC-MS	Tree shrew	Serum	7	Arachidonic acid (ofa) Taurocholic acid (chod) 2-octenoylcarnitine (aad)	[34]
	Cytomegalic Disease	HCMV	23	H-NMR	Human	Urine	5	3-hydroxybutyrate (bhca) 3-aminoisobutyrate (aad)	[35]
	Hepatitis E	HEV	44	H-NMR	Human	Plasma	4	Isoleucine (aa) Glycerol (sa) Acetone (ket)	[36]
		HEV	34	H-NMR	Human	Urine	7	1-methylnicotinamide (vbd) 1-methylhistidine (aad) 3-aminoisobutanoic acid (cad) Biopterin (pd) Adenosine (pn) Imidazole (dad) Salicyluric acid (bad)	[36]
	Simian AIDS	SIV	8	LC-MS	Monkey	CS-fluid	4	Carnitine (aa) Acylcarnitine (aad)	[37]

Chemical class abbreviations of biomarker VOMs: aa = amino acid; aad = amino acid deriv.; al = alcohol; as = amino sugar; asd = amino sugar deriv.; bad = benzoic acid deriv.; bcad = benzene carboxylic acid deriv.; bcd = benzene cresol deriv.; bd = benzene deriv.; bhca = beta-hydroxy carboxylic acid; cad = carboxylic acid deriv.; chod = cholesterol deriv.; dad = diazole deriv.; dca = dicarboxylic acid; ds = disaccharide; fad = fatty acid deriv.; fd = furan derivative; gsd = glycoside deriv.; had = heterocyclic amine deriv.; ket = ketone; ms = monosaccharide; ns = nucleoside; ofa = omega fatty acid; pad = primary amine deriv.; pca = phosphorylcholine deriv.; pd = pterin deriv.; pn = purine nucleoside; pud = purine deriv.; sa = sugar alcohol; sda = secondary amine; tca = tricarboxylic acid; ts = trisaccharide; vbd = vitamin B deriv.

various disease processes, produce biomarker metabolites that are usually derivative compounds of the unique set of metabolite intermediates produced by each respective pathway affected. For example, disease processes affecting amino acid metabolism produce amino acid derivatives specific to the particular amino acid metabolic pathway altered, whereas diseases affecting lipid metabolism tend to produce fatty acid derivatives. Diseases affecting the Kreb cycle pathways tend to produce various carboxylic acid (CA) derivatives, (*e.g.* benzene-CA, beta-hydroxy-CA, mono-CA, di-CA, or tri-CA) or organic acids, as VOMs. Similarly, disease effects on sugar metabolism give rise to mono-, di-, and tri-saccharide derivatives, or more oxidized sugar alcohol derivatives, whereas effects on nucleoside metabolism produce primarily purine derivatives.

Different categories of microbial pathogens cause different chemical effects on individual metabolic pathways often resulting in uniquely different types and classes of potential VOMs that may be identified as potential biomarkers associated with the unique diseases caused by individual pathogens. In other cases, the effects of disease processes by different types of microbial pathogens can give rise to some of the same biomarker metabolites, but usually in different VOM combinations and quantities, depending on the particular pathogen or type of disease each cause and the organ system affected.

4. BIOMARKER CATEGORIES

All biomarkers identified in association with specific health conditions, disease states, and physiological status which are affected by many measurable chemical parameters, may be divided into two main types: endogenous and exogenous biomarkers based on the origin of individual VOCs detected. Endogenous VOM biomarkers are those that originate within the body either from normal physiological pathways, from modified disease-related pathways, or from the metabolisms of invading microbes. Exogenous biomarkers are VOCs that originated from sources external to the body and entered into the body through inhalation, ingestion,

absorption through the skin, or some other means. Some have suggested that exogenous biomarkers cannot be direct indicators of infectious diseases, but might serve as disease-predisposition biomarkers [38, 39].

Numerous subcategories of endogenous and exogenous biomarkers have been identified through metabolomics and metabonomics that provide different types of information about a patient's physiological stages and overall state of health. Some of these other identified categories of biomarkers, including predisposition, disease, pathogen and gut-flora biomarkers, will be examined individually in the following discussions relative to their significance and nature in association with disease detection and diagnosis.

Disease-predisposition biomarkers are VOCs that originate from behavioral (often habitual), genetic or environmental factors or chemical exposures that can predispose individuals to certain types of diseases. Predisposition biomarkers can arise in individuals that are overweight or have high blood pressure, smokers, alcoholics, drug users, and those with oxidative, mental or physical stresses. For example, the appearance of specific biomarkers in the breath air of chronic smokers, particularly those suffering from epidermoid laryngeal carcinomas (ELC), were found to be different from breath biomarkers associated with the disease itself [40]. This study revealed four biomarker compounds related to cigarette consumption that suggested smoking is a predisposition to ELC disease. They found four predisposition biomarkers related to smoking including benzene, furaldehyde, 4-isobutyl-1-(1-dydroxyethyl)-benzene, and 2,3,5-trimethylhexane. Seven disease biomarkers were found in ELC patients who were nonsmokers in less advanced stages of the disease, and the most significant biomarkers identified were ethanol and 2-butanone.

Disease biomarkers are VOMs that result from alterations in normal metabolic pathways due to the disease process (pathogenesis). Some scientists include among disease biomarkers those metabolites produced and released directly by microbial pathogens themselves that have been closely correlated with the disease by etiological and metabolomic studies. Other scientists separate disease biomarkers from microbial metabolites, but include only metabolites originating from the host which are induced by or resulting directly from pathogenesis.

Pathogen biomarkers are mostly volatile metabolic markers produced directly by the unique biochemical pathways of microbial pathogens, whether bacterial, viral, fungal, protozoa, nematode, or schistosome origin. Many of these VOCs are secondary metabolites not required for normal cellular processes. In addition, each microbial species has a distinct, unique set of metabolic pathways for anabolic and catabolic processes that generate specific mixtures of species-specific VOCs that may be detected for diagnostic purposes. The distinct volatile biomarkers produced by six of the most abundant pathogenic bacteria causing human sepsis, including *Staphylococcus areus*, *Streptococcus pneumoniae*, *Entercoccus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli* were identified and evaluated recently for diagnostic accuracy in critically ill patients [10]. Species-specific and strain-specific biomarkers often provide information useful for more effective treatments against specific pathogen subtypes.

Gut-microflora biomarkers are VOMs that are produced by new or different microbes that arise or predominate in the gut due to perturbations of gut microbiota communities by pathogens or as a result of disease. These changes in bacteria present in the gut result from changes in pH, nutrients, or other chemical factors within the gut as a consequence of infectious diseases, particularly diseases affecting or occurring in the intestine that give rise to new types of VOCs representative of the different metabolic pathways utilized by these new gut microflora. A recent study showed that changes in the gut environment caused by *Salmonella typhimurium*-infection favored increased growth of different gut microflora that produced greater quantities of certain saccharides, such as melibiose and fucose moieties in the infected gut, which also favor growth of the pathogen in the gut during intestinal infection [17].

5. FACTORS AFFECTING BIOMARKER PRESENCE

There are numerous factors and variables that can affect VOC profiles derived from analysis of volatile metabolites in exhaled breath or the headspace of biofluids. Factors such as genetic predispositions, diet, exercise, stress, immune and infection status, drug use, smoking, alcohol consumption, overall physiological and health, and many other factors may affect metabolic processes that generate VOMs in the body [41]. These factors can influence the presence and relative quantities of non-invasive biomarkers used in early disease detection. Thus, attempts to standardize the conditions under which VOC profiles are examined is critical to properly identifying true biomarkers of disease. A recent study by Bikov [42] examined the effect of different breath parameters, such as exhalation flow and breath-hold, on levels of measurable gases in exhaled breath. They found that these exhalation parameters may significantly affect VOC levels (and resulting VOC profiles) from proton transfer reaction mass spectrometry (PTR-MS) analyses of expired air associated with lung diseases.

The human body produces and releases thousands of VOCs that have been identified from healthy human individuals and a comprehensive list was recently compiled in a compendium based on location and sources in the body [43]. The assemblage of all currently known VOCs produced by the body, collectively referred to as the volatome, were assigned CAS registry numbers and named according to a common convention. The components of this list were categorized based on chemical class or functionality. The results of this work led to some significant revelations. No ester VOCs were found in urine, but high numbers of esters were found in feces. Relatively few VOCs were found in blood compared to the large number of VOCs identified in the human breath. The large number of volatiles identified from skin was found to be related to the methodologies used for detection. Phillips [44] reported a list of the most prevalent VOCs in the breath of healthy humans. Broza [45] devised a new methodology for profiling body chemistry by analyzing the volatile fraction from various body fluids using gas chromatography-mass spectrometry (GC-MS) in combination with two cross-reactive e-noses, a 19-sensor organically

stabilized spherical gold nanoparticle (GNP) e-nose, and a 6-sensor random networks single-walled carbon nanotube (RN-SWCNT) e-nose. By simultaneous VOC-detection from breath and skin samples, they obtained complementary, non-correlated information of the body's volatile metabolites profile. They proposed that this methodology could provide more accurate monitoring of pathological changes in the body than information derived from a single body fluid. Information from these type studies have provided invaluable baseline information of healthy individuals that can be used to compare with VOCs produced by patients in various diseased states in order to better understand pathological mechanisms and processes.

6. METABOLOMICS OR ELECTRONIC-NOSE?

Some researchers have suggested that metabolomics is particularly useful for disease diagnosis and prognosis due to the high reproducibility of nuclear magnetic resonance (NMR)-based techniques, the utility in biomarker discovery and for investigating disease processes for a number of major diseases, the relatively low expensive on a per-sample cost basis, and the wide range of biofluid sample types that may be analyzed [11]. NMR and GC-MS metabolomic approaches also allow the detection of abnormal changes in NVOMs due to disease conditions. However, several important disadvantages of metabolomics methods have been identified such as the difficulty in achieving reliable diagnoses based on the quantification of relatively few biomarkers, the large variability that can arise from external stimuli (*e.g.* diet, lifestyle, physical activity, sample collection and preparation methods) not related to the disease condition, and the small number (<100) metabolites that can be detected with this approach. These problems have prompted many clinical diagnosticians and medical researchers with more applied objectives (workable POCT clinical procedures) to seek new methods that take into account all potential biomarkers as one complete disease signature or VOC profile in order to develop better approaches to using biomarker data in more reliable, timely, and effective ways.

Electronic-nose instruments and devices of numerous types and operational mechanisms have been tested for POCT clinical applications, including disease detection and diagnosis, based on the detection of complex gaseous VOC mixtures in sample headspace [1, 8, 12, 46]. VOCs generally are defined as organic compounds having molecular weights up to 300 Daltons and boiling points no greater than 250 °C at standard temperature and pressure (STP) [46]. E-nose instruments are ideal for profiling the VOC composition of complex gas mixtures because these devices identify and recognize aggregate patterns of VOCs present rather than identify individual compounds present in the sample mixture [7, 47]. Consequently, e-nose systems utilize and rely on pattern recognition algorithms and application-specific reference databases representing unique VOC patterns derived from the analysis of gaseous samples collected from patients with known diseases. The unique aroma signatures, derived from the aggregate outputs of sensor arrays, provide the patterns characteristic of the unique VOC mixtures present in breath and headspace samples taken from biofluids of diseased patients. Disease-specific e-nose databases of VOC patterns derived from samples of patients with known diseases provide diagnostic reference aroma signature patterns to which air samples from patients with unknown diagnoses may be compared.

Some distinct disadvantages of e-nose approaches to disease detection include the inability to detect changes in NVOMs (a limitation of only detecting changes in VOMs), variable physiological factors affecting VOC metabolite profiles of individual patients, the possible occurrence of multiple simultaneous diseases in the body, and variabilities in the body chemistries (composite metabolisms) of patients with the same disease. These variables require the use of application-specific reference databases (ASRDs) for e-nose recognition of each specific disease in order to overcome detection limitations. The construction of effective ASRDs (with sufficient discrimination power) should involve the input of numerous replication databases of healthy and diseased VOC-signature patterns from individual patients, representing the full range of patient-associated variability factors, in defining diseased *vs*. healthy metabolic states.

The metabolomics approach to disease detection relies on identified changes in the types and amounts of VOCs present in the sample. The metabolomics process of disease detection becomes quite complicated when numerous biomarkers are involved, requiring complex statistical analyses and mathematical models to determine the significance of the biomarkers used in detecting disease. Theoretically, the greater the number of disease-specific biomarkers identified, the greater the probability that the disease is present. However, this determination often requires very extensive data analysis and interpretation to arrive at a conclusive diagnosis. By contrast, the e-nose takes into account all types of disease-associated and pathogen biomarkers present and produces a signature by virtue of the sensor array output that generates a single aroma pattern that can be compared against known diagnostic signature patterns in disease-specific reference databases. Some nonspecific biomarker metabolites have been associated with different diseases, suggesting similar mechanisms of disease, although it is quite rare for there to be a large percentage overlap of all identified biomarkers for different diseases [8]. Recognized disease biomarkers, by definition, should be sufficiently specific and unique to particular diseases or very closely-related diseases. Thus, VOC signature patterns (aroma profiles), based on sensor array outputs in response to the complex mixture of all VOCs and biomarker metabolites present in the sample analyte, usually are sufficiently unique for e-nose discrimination of different diseases, particularly when using ASRDs.

Another advantage of e-nose analysis is the capability of taking into account differences in relative molar ratios of VOCs present in very complex gaseous mixtures by defining aroma signatures using ASRDs. For example, two separate complex mixtures containing exactly the same combination of VOCs but with different molar ratios of individual components, will result in different e-nose signature patterns as defined by the multisensor array [47]. Consequently, this capability of e-nose instruments to account for differences in the relative quantities of individual VOCs present in diagnostic samples (as a result of disease) covers one of the key functions of metabolomics analyses. Of course, e-nose sen-

sor arrays also measure differences in VOC components that are members of different chemical classes as determined by the range of sensitivity of each sensor in the sensor array. Individual sensors in the e-nose can be selected based on sensitivity to certain chemical classes and modified to target the range of biomarker metabolites most associated with particular diseases. This allows the development of application-specific e-nose instruments, utilizing ASRDs, designed to detect specific diseases which reduce costs and increases effectiveness of diagnosis for the particular disease the e-nose was designed to detect.

7. E-NOSE APPLICATIONS IN DISEASE DIAGNOSES

Electronic-nose devices, containing multisensory arrays composed of cross-reactive sensors, are particularly suitable for recognizing specific complex mixtures of VOC metabolites, such as noninvasive biomarkers present in exhaled air or in the headspace of biofluids, because these instruments are designed to detect unique aroma patterns associated with precise VOC mixtures rather than determine the exact chemical composition of the gaseous mixture [46]. E-nose devices can be designed for specific applications by careful and precise selection of sensor array components that are most sensitive and discriminating of VOCs from specific chemical classes [47]. Consequently, knowledge of the specific combination of biomarkers associated with a disease, once known, can be used to configure the instrument to very effectively detect a particular disease of interest, based on discrimination of sensory responses from normal or healthy controls. In this way, application-specific e-nose methods can be established to detect and diagnose specific diseases for which the instrument was specifically designed.

Discoveries of new potential clinical applications of electronic-nose devices have expanded dramatically since these instruments were first developed in the mid-1980s. E-nose devices have been tested for the detection of VOC patterns and biomarker signatures for numerous noninfectious diseases of the lungs, breast, bowels, colon, ovaries, prostate, and upper respiratory tract including asthma, cystic fibrosis (CF), inflammatory arthritis (IA), obstructive sleep apnoea (OSA), primary ciliary dyskinesia (PCD), and various types

Table 2. Recent testing of electronic-nose devices to detect VOC aroma signatures for noninfectious diseases.

Disease Name	Location	Sample Type	N=	E-nose Model	Sensor Type and No.[a]	Refs.
Asthma	Lung	Exhaled breath	40	Bloodhound 114	CP 14	[48]
BAD	Bowel	Urine	110	Fox 4000	MOS 18	[49]
Cancer	Breast	Exhaled breath	22	Experimental	GNP 14	[50]
	Breast	Exhaled breath	244	BreathLink	GC + SAW 1	[51]
	Colon	Exhaled breath	26	Experimental	GNP 14	[50]
	Colon	Fecal	157	Cyranose 320	CP 32	[52]
	Lung	Exhaled breath	30	Experimental	GNP 14	[50]
	Lung	Cell lines	36	Experimental	GNP 18	[53]
	Lung	Exhaled breath	30	LibraNose	QMB 8	[54]
	Lung	Exhaled breath	229	Experimental	CSA 24	[55]
	Ovaries	Ovarian tissue	162	TSG 2600	MOS 16	[56]
	Prostate	Exhaled breath	18	Experimental	GNP 14	[50]
	Prostate	Urine	74	ChemPro 100i	IMS 1	[57]
CF	Upper airway	Exhaled breath	85	Cyranose 320	CP 32	[58]
	Upper airway	Exhaled breath	48	Cyranose 320	CP 32	[2]
IA	Joints	Exhaled breath	60	Cyranose 320	CP 32	[59]
OSA	Upper airway	Exhaled breath	60	Cyranose 320	CP 32	[60]
	Upper airway	Exhaled breath	36	Cyranose 320	CP 32	[61]
PCD	Upper airway	Exhaled breath	42	Cyranose 320	CP 32	[58]
	Upper airway	Exhaled breath	48	Cyranose 320	CP 32	[2]

[a]Sensor type abbreviations: CSA = colorimetric sensor array; CP = conducting polymer; GC = gas chromatograph, GNP = gold nanoparticle; IMS = ion-mobility spectrometry; MOS = metal oxide semiconductors; QMB = quartz crystal microbalance; and SAW = surface acoustic wave.

of cancer (Table **2**). These tests have involved analyses of exhaled breath, fecal, urine, tissue samples, and diseased cell lines as sources of VOCs for e-nose analyses. The specific types of e-nose technologies used for non-infectious disease detection have included primarily conducting polymer (CP), gold nanoparticle (GNP), metal oxide semiconductors (MOS), quartz crystal microbalance (QMB), and surface acoustic wave (SAW) e-nose sensor types.

Electronic-nose devices also have been tested for the detection and diagnosis of infectious diseases caused by microbial pathogens (bacterial, fungal, and protozoa), based on analyses of VOC patterns and unique biomarker signatures of headspace volatiles from blood, exhaled breath, urine, and microbial cultures using carbon nanofiber (CNF), CP, and MOS sensor arrays (Table **3**). The complex VOC signatures derived from exhaled breath and headspace volatiles derived from biofluids sampled from patients with infectious disease contain VOMs derived from the unique metabolic pathways of the microbial pathogens themselves and from alterations in human-host cellular pathways as a consequence of specific microbial disease processes. Thus, infectious diseases tend to produce more complex and various types of VOMs (more diverse chemical classes) than non-infectious diseases due to the added components of VOM products from the unique metabolic pathways of microbial pathogens. Most VOC biomarker metabolites of microbes have been identified for bacterial pathogens, yet relatively few biomarkers have been identified for pathogenic fungi [70].

CONCLUSION

Metabolomic methods and approaches will continue to be utilized to help identify key metabolites as potential disease biomarkers for major diseases, including the most significant chemical components of metabolic fingerprints, and for elucidating the mechanisms of disease processes. These are the greatest strengths of metabolic approaches which are most useful as basic research tools for disease discovery and understanding mechanisms of disease processes and their effects on normal physiological pathways involved in cellular processes. Nevertheless, the utility of metabolomic approaches for real-time diagnoses of diseases in POCT clinical situations are somewhat limited due to the time requirement for such sophisticated chemical analyses to be completed and the inability of analyzing diagnostic samples as aggregate or whole complex VOC mixtures to produce composite volatile signatures or aroma profiles. The metabolic response of human hosts to different pathogens and diseases often overlaps or is sufficiently onerous or complex to render metabolomic analysis (of relatively few biomarker metabolites) insufficient for diagnoses. A much simpler and easier approach using electronic-nose devises is more appropriately designed for rapid analyses of complex gaseous mixtures, such as in exhaled breath samples to produce "breathprints" or unique volatile patterns, and can be used to identify specific diseases and provide medical personnel with timely information reliable for accurate diagnoses without having to send diagnostic samples to the laboratory for chemical and culture analysis. Great strides recently have been made in developing e-nose technologies for rapid POCT for diagnoses in clinical settings for numerous diseases. The many types of e-nose technologies will continue to be improved with more clinical testing and as procedures for use of these instruments are standardized and refined for a wide range of diseases and clinical applications.

Table 3. Recent testing of electronic-nose devices to detect VOC aroma signatures for infectious diseases.

Disease Type	Disease Name	Pathogen	Location	Sample Type	N=	E-nose Model	Sensor Type and No.[a]	Refs.
Bacterial	Microbial infections	Various	Upper airway	Exhaled breath	192	Cyranose 320	CP 32	[62]
	Microbial infections	Various	Foot	Cultures	350-750	Cyranose 320	CP 32	[63]
	Microbial infections	Various	Urinary	Cultures	101	IMS Cell-eNose	IMS 8, MOS 6	[64]
	Salmonellosis	*Salmonella spp.*	Blood	Flagellin	$>10^3$	DR-ADF	MOS-BS	[65]
	Tuberculosis	*M. tuberculosis*	Lung	Exhaled breath	194	DiagNose	MOS 12	[66]
	Urinary tract infections	*Escherichia coli, Proteus spp., Staphylococcus spp.*	Urinary	Urine	25, 45	Bloodhound BH114	CP 14	[67]
Fungal	Aspergillosis	*Aspergillus spp.*	Lung	Exhaled breath	46	Cyranose 320	CP 32	[68]
Protozoa	Malaria	*P. falciparum*	Blood	Biological antigen solution	us[b]	Experimental	CNF-BS	[69]

[a]Sensor type abbreviations: CP = conducting polymer; CNF-BS = carbon nanofiber biosensor; IMS = ion-mobility spectrometry; MOS = metal oxide semiconductors.

[b]us = unspecified.

LIST OF ABBREVIATIONS

ASRDs	=	Application-Specific Reference Databases
BAD	=	Bile Acid Diarrhea
BS	=	Bio Sensor
CE	=	Capillary Electrophoresis
CF	=	Cystic Fibrosis
CHBV	=	Chronic Hepatitis B Virus
CNF	=	Carbon Nanofiber
CP	=	Conducting Polymer
CPMG	=	Carr-Purcell-Meiboom-Gill (NMR)
CSA	=	Colorimetric Sensor Array
E-nose	=	Electronic Nose
ELC	=	Epidermoid Laryngeal Carcinomas
GC-MS	=	Gas Chromatography-Mass Spectrometry
GNP	=	Gold Nanoparticle
HCMV	=	Human Cytomegalovirus
HCV	=	Hepatitis C Virus
HEV	=	Hepatitis E Virus
IA	=	Inflammatory Arthritis
IMS	=	Ion-Mobility Spectrometry
LC	=	Liquid Chromatography
MOS	=	Metal Oxide Semiconductors
NMR	=	Nuclear Magnetic Resonance
NVOMs	=	Non-Volatile Organic Metabolites
OSA	=	Obstructive Sleep Apnoea
PCD	=	Primary Ciliary Dyskinesia
P-MRS	=	Phosphorus Magnetic Resonance Spectroscopy
POCT	=	Point-of-Care Testing
PTR-MS	=	Proton Transfer Reaction Mass Spectrometry
QMB	=	Quartz Crystal Microbalance
RN-SWCNT	=	Random Networks Single-Walled Carbon Nanotube
SAW	=	Surface Acoustic Wave
SIV	=	Simian Immunodeficiency Virus
STP	=	Standard Temperature and Pressure
UPLC	=	Ultra-Performance Liquid Chromatography
VOCs	=	Volatile Organic Compounds
VOMs	=	Volatile Organic Metabolites

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supportive material is available on the publisher's website along with the published article.

REFERENCES

[1] Wilson, A.D. Recent progress in the design and clinical development of electronic-nose technologies. *Nanobiosens. Dis. Diagnos.*, **2016**, *5*, 15-27.

[2] Paff, T.; van der Schee, M.P.; Daniels, J.M.A.; Pals, G.; Postmus, P.E.; Sterk, P.J.; Haarman, E.G. Exhaled molecular profiles in the assessment of cystic fibrosis and primary ciliary dyskinesia. *J. Cystic Fibrosis*, **2013**, *12*, 454-460.

[3] Wilson, A.D. Theoretical and practical considerations for teaching diagnostic electronic-nose technologies to clinical laboratory technicians. *Proc. Soc. Behav. Sci.*, **2012**, *31*, 262-274.

[4] Wilson, A.D. Advanced methods for teaching electronic-nose technologies to diagnosticians and clinical laboratory technicians. *Proc. Soc. Behav. Sci.*, **2012**, *46*, 4544-4554.

[5] Li, H.; Tang, H.; Wang, Y. Advances in metabonomics on infectious diseases. *Curr. Metabolomics*, **2013**, *1*, 318-334.

[6] Heger, M. Fingerprinting disease. *Discover*, **2016**, *May*, 28-29.

[7] Chan, D.K.; Leggett, C.L.; Wang, K.K. Diagnosing gastrointestinal illnesses using fecal headspace volatile organic compounds. *World J. Gastroenterol.,* **2016**, *22*, 1639-1649.

[8] Wilson, A.D. Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath. *Metabolites*, **2015**, *5*, 140-163.

[9] Wilson, A.D. Electronic-nose applications in forensic science and for analysis of volatile biomarkers in the human breath. *J. Forensic Sci. Criminol.*, **2014**, *1*, S103.

[10] Bos, L.D.J.; Sterk, P.J.; Schultz, M.J. Volatile metabolites of pathogens: A systematic review. *PLoS Pathog.*, **2013**, *9*, e1003311.

[11] Emwas, A.M.; Salek, R.M. NMR-based metabolomics in human disease diagnosis: applications, limitations, and recommendations. *Metabolomics*, **2013**, *9*, 1048-1072.

[12] Wilson, A.D., Baietto, M. Advances in electronic-nose technologies developed for biomedical applications. *Sensors*. **2011**, *11*, 1105-1176.

[13] Gao, X.X.; Ge, H.M.; Zheng, W.F.; Tan, R.X. NMR-based metabonomics for detection of *Helicobacter pylori* infection in gerbils: Which is more descriptive. *Helicobacter*, **2008**, *13*, 103-111.

[14] du Preez, I.; Loots, D.T. New sputum metabolite markers implicating adaptations of the host to *Mycobacterium tuberculosis*, and vice versa. *Tuberculosis*, **2013**, *93*, 330-337.

[15] Weiner, J., III; Parida, S.K.; Maertzdorf, J.; Black, G.F.; Repsilber, D.; Telaar, A.; Mohney, R.P.; Arndt-Sullivan, C.; Ganoza, C.A.; Fae, K.C.; Walzl, G.; Kaufmann, S.H.E. Biomarkers of inflammation, immunosuppression and stress with active disease are revealed by metabolomic profiling of tuberculosis patients. *PloS One*, **2012**, *7*, e40221.

[16] Che, N.; Cheng, J.; Li, H.; Zhang, Z.; Zhang, X.; Ding, Z.; Dong, F.; Li, C. Decreased serum 5-oxoproline in TB patients is associated with pathological damage of the lung. *Clin. Chim. Acta*, **2013**, *423*, 5-9.

[17] Kaiser, B.L.D.; Li, J.; Sanford, J.A.; Kim, Y.M.; Kronewitter, S. R.; Jones, M.B.; Peterson, C.T.; Peterson, S.N.; Frank, B.C.; Purvine, S.O.; Brown, J. N.; Metz, T.O.; Smith, R.D.; Heffron, F.; Adkins, J.N.A multi-omic view of host-pathogen-commensal interplay in *Salmonella*-mediated intestinal infection. *PloS One*, **2013**, *8*, e67155.

[18] Slupsky, C.M.; Cheypesh, A.; Chao, D.V.; Fu, H.; Rankin, K.N.; Marrie, T.J.; Lacy, P. *Streptococcus pneumoniae* and *Staphylococcus aureus* pneumonia induce distinct metabolic responses. *J. Proteome Res.*, **2009**, *8*, 3029-3036.

[19] Hoerr, V.; Zbytnuik, L.; Leger, C.; Tam, P.P.C.; Kubes, P.; Vogel, H.J. Gram-negative and Gram-Positive bacterial infections give rise to a different metabolic response in a mouse model. *J. Proteome Res.*, **2012**, *11*, 3231-3245.

[20] Slupsky, C.M.; Rankin, K.N.; Fu, H.; Chang, D.; Rowe, B.H.; Charles, P.G.P.; McGeer, A.; Low, D.; Long, R.; Kunimoto, D.; Sawyer, M.B.; Fedorak, R.N.; Adamko, D.J.; Saude, E.J.; Shah, S. L.; Marrie, T.J. Pneumococcal pneumonia: Potential for diagnosis through a urinary metabolic profile. *J. Proteome Res.*, **2009**, *8*, 5550-5558.

[21] Chambers, S.T.; Bhandari, S.; Scott-Thomas, A.; Syhre, M. Novel diagnostics: Progress toward a breath test for invasive *Aspergillus fumigatus*. *Med. Mycol.*, **2011**, *49*, S54-S61.

[22] Parekh, S.B.; Bubb, W.A.; Hunt, N.H.; Rae, C. Brain metabolic markers reflect susceptibility status in cytokine gene knockout mice with murine cerebral malaria. *Int. J. Parasitol.*, **2006**, *36*, 1409-1418.

[23] Li, J.V.; Wang, Y.; Saric, J.; Nicholson, J.K.; Dirnhofer, S.; Singer, B.H.; Tanner, M.; Wittlin, S.; Holmes, E.; Utzinger, J. Global metabolic responses of NMRI mice to an experimental *Plasmodium berghei* infection. *J. Proteome Res.*, **2008**, *7*, 3948-3956.

[24] Olszewski, K.L.; Morrisey, J.M.; Wilinski, D.; Burns, J.M.; Vaidya, A.B.; Rabinowitz, J.D.; Linas, M. Host-parasite interactions revealed by *Plasmodium falciparum* metabolomics. *Cell Host Microbe*, **2009**, *5*, 191-199.

[25] Sengupta, A.; Ghosh, S.; Basant, A.; Malusare, S.; Johri, P.; Pathak, S.; Sharma, S.; Sonawat, H.M. Global host metabolic response to *Plasmodium vivax* infection: A H-1 NMR based urinary metabonomic study. *Malar. J.*, **2011**, *10*, 384.

[26] Li, J.V.; Saric, J.; Wang, Y.; Utzinger, J.; Holmes, E.; Balmer, O. Metabonomic investigation of single and multiple strain *Trypanosoma brucei* infections. *Am. J. Trop. Med. Hyg.*, **2011**, *84*, 91-98.

[27] Wang, Y.; Utzinger, J.; Saric, J.; Li, J.V.; Burckhardt, J.; Dirnhofer, S.; Nicholson, J.K.; Singer, B.H.; Brun, R.; Holmes, E. Global metabolic responses of mice to *Trypanosoma brucei* infection. *Proc. Natl. Acad. Sci. USA.*, **2008**, *105*, 6127-6132.

[28] Wu, J.; Xu, W.; Ming, Z.; Dong, H.; Tang, H.; Wang, Y. Metabolic changes reveal the development of schistosomiasis in mice. *PLoS Negl. Trop. Dis.*, **2010**, *4*, e807.

[29] Garcia-Perez, I.; Earll, M.E.; Angulo, S.; Barbas, C.; Legido- Quigley, C. Chemometric analysis of urine fingerprints acquired by liquid chromatography-mass spectrometry and capillary electrophoresis: Application to the schistosomiasis mouse model. *Electrophoresis*, **2010**, *31*, 2349-2355.

[30] Garcia-Perez, I.; Angulo, S.; Utzinger, J.; Holmes, E.; Legido-Quigley, C.; Barbas, C. Chemometric and biological validation of a capillary electrophoresis metabolomic experiment of *Schistosoma mansoni* infection in mice. *Electrophoresis*, **2010**, *31*, 2338-2348.

[31] Li, J.V.; Saric, J.; Wang, Y.; Keiser, J.; Utzinger, J.; Holmes, E. Chemometric analysis of biofluids from mice experimentally infected with *Schistosoma mansoni*. *Parasit. Vectors*, **2011**, *4*, 179.

[32] Yu, K.; Sheng, G.P.; Sheng, J.F.; Chen, Y.M.; Xu, W.; Liu, X.L.; Cao, H.C.; Qu, H.B.; Cheng, Y.Y.; Li, L.J. A metabonomic investigation on the biochemical perturbation in liver failure patients caused by hepatitis b virus. *J. Proteome Res.*, **2007**, *6*, 2413-2419.

[33] Zhang, A.; Sun, H.; Han, Y.; Yan, G.; Wang, X. Urinary metabolic biomarker and pathway study of hepatitis B virus infected patients based on UPLC-MS system. *PloS One*, **2013**, *8*, e64381.

[34] Sun, H.; Zhang, A.; Yan, G.; Piao, C.; Li, W.; Sun, C.; Wu, X.; Li, X.; Chen, Y.; Wang, X. Metabolomic analysis of key regulatory metabolites in Hepatitis C virus-infected tree shrews. *Mol. Cell. Proteomics*, **2013**, *12*, 710-719.

[35] Fanos, V.; Locci, E.; Noto, A.; Lazzarotto, T.; Manzoni, P.; Atzori, L.; Lanari, M. Urinary metabolomics in newborns infected by human cytomegalovirus: A preliminary investigation. *Early Hum. Dev.*, **2013**, *89*, S58-S61.

[36] Munshi, S.U.; Taneja, S.; Bhavesh, N.S.; Shastri, J.; Aggarwal, R.; Jameel, S. Metabonomic analysis of hepatitis E patients shows deregulated metabolic cycles and abnormalities in amino acid metabolism. *J. Viral Hepat.,* **2011**, *18*, E591-E602.

[37] Wikoff, W.R.; Pendyala, G.; Siuzdak, G.; Fox, H.S. Metabolomic analysis of the cerebrospinal fluid reveals changes in phospholipase expression in the CNS of SIV-infected macaques. *J. Clin. Invest.* **2008**, *118*, 2661-2669.

[38] Nicholson, J.K.; Lindon, J.C.; Holmes, E. 'Metabonomics'. Understanding the metabolic responses of living systems to pathophysiological stimuli *via* multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*, **1999**, *29*, 1181-1189.

[39] Tang, H.R.; Wang, Y.L. Metabonomics: A revolution in progress. *Prog. Biochem. Biophys.*, **2006**, *33*, 401-417.

[40] Garcia, R.A.; Morales, V.; Martín, S.; Vilches, E.; Toledano, A. Volatile organic compounds analysis in breath air in healthy volunteers and patients suffering epidermoid laryngeal carcinomas. *Chromatographia.*, **2014**, *77*, 501-509.

[41] Whittie, C.L.; Fakharzadeh, S.; Eades, J.; Preti, G. Human breath odors and their use in diagnosis. *Ann. N.Y. Acad. Sci.*, **2007**, *1098*, 252-266.

[42] Bikov, A.; Paschalaki, K.; Logan-Sinclair, R.; Horvath, I.; Kharitonov, S.A.; Barnes, P.J.; Usmani, O.S.; Paredi, P. Standardised exhaled breath collection for the measurement of exhaled volatile organic compounds by proton transfer reaction mass spectrometry. *BMC Pulmon. Med.*, **2013**, *13*, 43.

[43] de Lacy Costello, B.; Amann, A.; Al-Kateb, H.; Flynn, C.; Filipiak, W.; Khalid, T.; Osborne, D.; Ratcliffe, N.M. A review of the volatiles from the healthy human body. *J. Breath Res.*, **2014**, *8*, 014001.

[44] Phillips, M.; Cataneo, R.N.; Chaturvedi, A.; Kaplan, P.D.; Libardoni, M.; Mundada, M.; Patel, U.; Zhang, X. Detection of an extended human volatome with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. *PLoS One*, **2013**, *8*, e75274.

[45] Broza, Y.Y.; Zuri, L.; Haick, H. Combined volatolomics for monitoring of human body chemistry. *Sci. Rep.*, **2014**, *4*, 4611.

[46] Wilson, A.D.; Baietto, M. Applications and advances in electronic-nose technologies. *Sensors*, **2009**, *9*, 5099-5148.

[47] Wilson, A.D.; Lester, D.G.; Oberle, C.S. Development of conductive polymer analysis for the rapid detection and identification of phytopathogenic microbes. *Phytopathology*, **2004**, *94*, 419-431.

[48] Montuschi, P.; Mores, N.; Trove, A.; Mondino, C.; Barnes, P.J. The electronic nose in respiratory medicine. *Respiration*, **2013**, *85*, 72-84.

[49] Covington, J.A.; Westenbrink, E.W.; Ouaret, N.; Harbord, R.; Bailey, C.; O'Connell, N.; Cullis, J.; Williams, N.; Nwokolo, C.U.; Bardhan, K.D.; Arasaradnam, R.P. Application of a novel tool for diagnosing bile acid diarrhoea. *Sensors*, **2013**; *13*, 11899-11912.

[50] Peng, G.; Hakim, M.; Broza, Y.Y.; Billan, S.; Abdah-Bortnyak, R.; Kuten, A.; Tisch, U.; Haick, H. *Brit. J. Cancer*, **2010**, *103*, 542-551.

[51] Phillips, M.; Beatty, J.D.; Cataneo, R.N.; Huston, J.; Kaplan, P.D.; Lalisang, R.I.; Lambin, P.; Lobbes, M.B.; Mundada, M.; Pappas, N.; Patel, U. Rapid point-of-care breath test for biomarkers of breast cancer and abnormal mammograms. *PLoS One*, **2014**, *9*, e90226.

[52] de Meij, T.G.; Larbi, I.B.; van der Schee, M.P.; Lentferink, Y.E.; Paff, T.; Terhaar Sive Droste, J.S.; Mulder, C.J.; van Bodegraven, A.A.; de Boer, N.K. Electronic nose can discriminate colorectal carcinoma and advanced adenomas by fecal volatile biomarker analysis: proof of principle study. *Int. J. Cancer*, **2014**, *134*, 1132–1138.

[53] Barash, O.; Peled, N.; Tisch, U.; Bunn, P.A.; Hirsh, F.R.; Haick, H. Classification of lung cancer histology by gold nanoparticle sensors. *Nanomed. NBM*, **2012**, *8*, 580-589.

[54] Capuano, R.; Santonico, M.; Pennazza, G.; Ghezzi, S.; Martinelli, E.; Roscioni, C.; Lucantoni, G.; Galluccio, G.; Paolesse, R.; Di Natale, C.; D'Amico, A. The lung cancer breath signature: A comparative analysis of exhaled breath and air sampled from inside the lung. *Sci. Rep.*, **2015**, *5*, 16491.

[55] Mazzone, P.J.; Wang, X.F.; Xu, Y.; Mekhail, T.; Beukemann, M.C.; Na, J.; Kemling, J.W.; Suslick, K.S.; Sasidhar, M. Exhaled breath analysis with a colorimetric sensor array for the identification and characterization of lung cancer. *J. Thorac. Oncol.* **2012**, *7*, 137-142.

[56] Chilo, J.; Horvath, G.; Lindblad, T.; Olsson, R.; Redeby, J.; Roeraade, J. A flexible electronic nose for ovarian carcinoma diagnosis in real time. Proceedings of the Real Time Conference 16th IEEE-NPSS; May 10-15, 2009; Beijing, China. New York: IEEE; 2009.

[57] Roine, A.; Veskimae, E.; Tuokko, A.; Kumpulainen, P.; Koskimaki, J.; Keinanen, T.A.; Hakkinen, M.R.; Vespalainen, J.; Paa-

vonen, T.; Lekkala, J.; Lehtimaki, T.; Tammela, T.L.; Oksala, N.K.J. Detection of prostate cancer by an electronic nose: A proof of principle study. *J. Urol.*, **2014**, *192*, 230-235.

[58] Joensen, O.; Paff, T.; haarman, E.G.; Skovgaard, I.M.; Jensen, P.O.; Bjarnsholt, T.; Nielsen, K.G. Exhaled breath analysis using electronic nose in cystic fibrosis and primary ciliary dyskinesia patients with chronic pulmonary infections. *PLoS One*, **2014**, *9*, e115584.

[59] Brekelmans, J.P.; Fens, N.; Brinkman, P.; Bos, L.D.; Sterk, P.J.; Tak, P.P.; Gerlag, D.M. Smelling the diagnosis: The electronic nose as diagnostic tool in inflammatory arthritis. A case-reference study. *PLoS One*, **2016**, *11*, e0151715.

[60] Greulich, T.; Hattesohl, A.; Grabisch, A.; Koepke, J.; Schmid, S.; Noeske, S.; Nell, C.; Wencker, M.; Jorres, R.A.; Vogelmeier, C.F.; Kohler, U.; Koczulla, A.R. Detection of obstructive sleep apnoea by an electronic nose. *Eur. Respir. J.*, **2013**, *42*, 145-155.

[61] Kunos, L.; Bikov, A.; Lazar, Z.; Korosi, B.Z.; Benedek, P.; Losonezy, G.; Horvath, I. Evening and morning exhaled volatile compound patterns are different in obstructive sleep apnoea with electronic nose. *Sleep Breath*, **2015**, *19*, 247-253.

[62] Shafiek, H.; Florentino, F.; Merino, J.L.; Lopez, C.; Oliver, A.; Segura, J.; de Paul, I.; Sibila, O.; Agusti, A.; Cosio, B.G. Using the electronic nose to identify airway infection during COPD exacerbations. *PLoS One*, **2015**, *10*, e0135199.

[63] Yusuf, N.; Zakaria, A.; Omar, M.I.; Shakaff, A.Y.; Masnan, M.J.; Kamarudin, L.M.; Abdul Rahim, N.; Zakaria, N.Z.; Abdullah, A.A.; Othman, A.; Yasin, M.S. *In-vitro* diagnosis of single and poly microbial species targeted for diabetic foot infection using e-nose technology. *BMC Bioinform.*, **2015**, *16*, 158-169.

[64] Roine, A.; Saviauk, T.; Kumpulainen, P.; Karjalainen, M.; Tuokko, A.; Aittoniemi, J.; Vuento, R.; Lekkala, J.; Lehtimaki, T.; Tammela, T.L.; Oksala, N.K.J. Rapid and accurate detection of urinary pathogens by mobile IMS-based electronic nose: A proof-of-principle study. *PLoS One*, **2014**, *9*, e114279.

[65] Bahadoran, M.; Noorden, A.F.A.; Chaudhary, K.; Mohajer, F.S.; Aziz, M.S.; Hashim, S.; Ali, J.; Yupapin, P. Modeling and analysis of a microresonating biosensor for detection of *Salmonella* bacteria in human blood. *Sensors*, **2014**, *14*, 12885-12899.

[66] Bruins, M.; Rahim, Z.; Bos, A.; van de Sande, W.W.J.; Endtz, H.P.; van Belkum, A. Diagnosis of active tuberculosis by e-nose analysis of exhaled air. *Tuberculosis*, **2013**, *93*, 232-238.

[67] Pavlou, A.K.; Magan, N.; McNulty, C.; Jones, J.; Sharp, D.; Brown, J.; Turner, A.P. Use of an electronic nose system for diagnoses of urinary tract infections. *Biosens. Bioelectron.*, **2002**, *17*, 893-899.

[68] de Heer, K.; van der Schee, M.P.; Zwinderman, K.; van den Berk, I.A.H.; Visser, C.E.; van Oers, R. Electronic nose technology for detection of invasive pulmonary Aspergillosis in prolonged chemotherapy-induced neutropenia: A proof-of-principle study. *J. Clin. Microbiol.*, **2013**, *51*, 1490-1495.

[69] Gikunoo, E.; Abera A.; Woldesenbet E. A novel carbon nanofibers grown on glass microballoons immunosensor: A tool for early diagnosis of malaria. *Sensors*, **2014**, *14*, 14686-14699.

[70] Lemfack, M.C.; Nickel, J.; Dunkel, M.; Preissner, R.; Piechulla, B. mVOC: A database of microbial volatiles. *Nucleic Acids Res.*, **2014**, *42*, D744-748.