AP&T Alimentary Pharmacology and Therapeutics

Review article: next generation diagnostic modalities in gastroenterology – gas phase volatile compound biomarker detection

R. P. Arasaradnam*,§, J. A. Covington†, C. Harmston‡ & C. U. Nwokolo§

*Clinical Sciences Research Institute, University of Warwick, Coventry, UK.
†School of Engineering, University of Warwick, Coventry, UK.
‡Department of Colorectal Surgery, University Hospital Coventry & Warwickshire, Coventry, UK.
§Department of Gastroenterology, University Hospital Coventry & Warwickshire, Coventry, UK.

Correspondence to:
Prof. C. U. Nwokolo, Department of Gastroenterology, University Hospital Coventry & Warwickshire, Clifford Bridge Road, Coventry CV2 2DX, UK.
E-mail: chukanwokolo@compuserve.com

Publication data
Submitted 21 September 2013
First decision 8 October 2013
Resubmitted 21 January 2014
Accepted 23 January 2014
EV Pub Online 20 February 2014

This commissioned review article was subject to full peer-review and the authors received an honorarium from Wiley, on behalf of AP&T.

SUMMARY

Background
The detection of airborne gas phase biomarkers that emanate from biological samples like urine, breath and faeces may herald a new age of non-invasive diagnostics. These biomarkers may reflect status in health and disease and can be detected by humans and other animals, to some extent, but far more consistently with instruments. The continued advancement in micro and nanotechnology has produced a range of compact and sophisticated gas analysis sensors and sensor systems, focussed primarily towards environmental and security applications. These instruments are now increasingly adapted for use in clinical testing and with the discovery of new gas volatile compound biomarkers, lead naturally to a new era of non-invasive diagnostics.

Aim
To review current sensor instruments like the electronic nose (e-nose) and ion mobility spectroscopy (IMS), existing technology like gas chromatography-mass spectroscopy (GC-MS) and their application in the detection of gas phase volatile compound biomarkers in medicine – focussing on gastroenterology.

Methods
A systematic search on Medline and Pubmed databases was performed to identify articles relevant to gas and volatile organic compounds.

Results
E-nose and IMS instruments achieve sensitivities and specificities ranging from 75 to 92% in differentiating between inflammatory bowel disease, bile acid diarrhoea and colon cancer from controls. For pulmonary disease, the sensitivities and specificities exceed 90% in differentiating between pulmonary malignancy, pneumonia and obstructive airways disease. These sensitivity levels also hold true for diabetes (92%) and bladder cancer (90%) when GC-MS is combined with an e-nose.

Conclusions
The accurate reproducible sensing of volatile organic compounds (VOCs) using portable near-patient devices is a goal within reach for today's clinicians.

Aliment Pharmacol Ther 2014; **39:** 780–789

doi:10.1111/apt.12657

INTRODUCTION

There is now a gradual paradigm shift with emphasis towards near-to-patient diagnosis using non-invasive methods. One important technique is the detection of airborne gas phase biomarkers emanating from human biological waste material including urine, sweat, breath and stool.

Clinicians have been using their own sense of smell as a diagnostic tool for centuries. Hippocrates himself suggested a patient's odour could lead to the diagnosis of their malaise. In his treatise on breath odour and disease, he described foetor oris and hepaticus. It has also been reported that he practised pouring human sputum on hot coals to diagnose *Mycobacterium Tuberculosis* (TB) on the basis of the foul odour that was emanated (http://en.wikipedia.org/wiki/Hippocratic_Corpus). Physicians can detect the sweet smell of diabetic ketoacidosis and the rancid odour of *C. difficile* stools. For those bequeathed with the gene to detect the bitter almond smell of cyanide, this may confer a survival advantage. In 1971, Nobel laureate Linus Pauling took this further by describing the complex mixture of volatile compounds (~250 compounds) present in breath and urine.[1] There are also reports that canines can be trained to detect early stages of disease.[2–4] We believe that animals use their sense of smell to detect gases and VOCs, and replicating this ability electronically is the key to unlocking advances in *ex vivo* testing.

The available technology has often been ahead of the clinical need, but greater awareness and collaboration with bioengineering have brought the two disciplines much closer. Markers that hold the most promise for this type of technology (and gaining credence) are associated with the detection of gas phase molecules present at room temperature. There is now an emerging portfolio of evidence in the literature citing the ability to detect diseases using various aroma scanning devices. These devices have been used to detect lung and skin cancers, wound infections, some ENT diseases, bacterial infections including MRSA and *C. difficille*, metabolic disorders, hypoxic states like asthma, COPD and bronchitis and even recreational drug use.[5, 6]

This review assesses this rapidly developing technology with focus on its application in gastroenterology and makes predictions as to where the future lies with devices that bring non-invasive diagnosis closer to the patient.

Analytical instruments in gas phase volatile compound detection

There are several available analytical methods that can be used to detect volatile organic compounds (VOCs), with gas chromatography (GC)/Mass Spectrometry (MS) being considered the gold standard. Although efficient at undertaking this role, the cost, bulkiness and operating overhead make them an unlikely routine diagnostic tool. Whilst miniaturised GCs have been under development from the mid 1970s, they have yet to find commercial success. This may be due to the combined difficulty in creating a stable coating and poor compatibility with existing GC equipment. Due to the biological origin of the VOCs, it is likely that they will contain a variable mix of compounds with different molecular weights. Gas phase biomarker molecules exist in small quantities and differing ratios, thus requiring highly adaptive instruments to rapidly capture and detect these biomarkers. One such instrument that can achieve this requirement, practical and economically viable is the 'electronic nose'.

The term electronic nose is broad – referring to a method rather than specific sensor technology. Such instruments do not detect every single chemical component but, like the human olfactory system, attempt to identify patterns in an array of nonspecific sensors. A traditional electronic nose is formed of an array of 8–32 different chemical sensors. These sensors are broadly tuned to different chemical groups including alcohols, ketones and low pressure gases. When the air above the biological sample (the 'headspace') is injected into the e-nose, each sensor response is unique within the array. A feature of this response, for example the maximum change, is then extracted and used to train the instrument using a pattern recognition engine (usually some form of neural network). Thus, it is possible to teach the instrument to recognise a range of different conditions. If the instrument is presented with a sample from the same disease group, the response pattern from the sensors is repeated and the instrument is able to identify the condition – as shown in Figure 1.

Electronic noses have been created using a range of technologies including carbon black composite polymers (Cyrano 320; Sensigent, Milwaukee, WI, USA), semi-conducting metal oxide chemoresistors (Fox 4000/3000/2000 AlphaMOS; Toulouse, France; PEN3), Airsense Analytical, (Schwerin, Germany), E-nose (E-nose company, Eveleigh, NSW, Australia), polymer-coated quartz crystal microbalances (MOSES II; GSG-Analytical, Brushal, Germany), optical dyes (BAI; Metabolomix, West Palm Beach, FL, USA), Gas FETs (NST3320; Applied Sensors, Reutlingen, Germany) and electrochemical sensors[5] (Tetra:3; Crowcon, Abingdon, UK).

However, more recent technologies have also now been classified under 'electronic nose' category. These

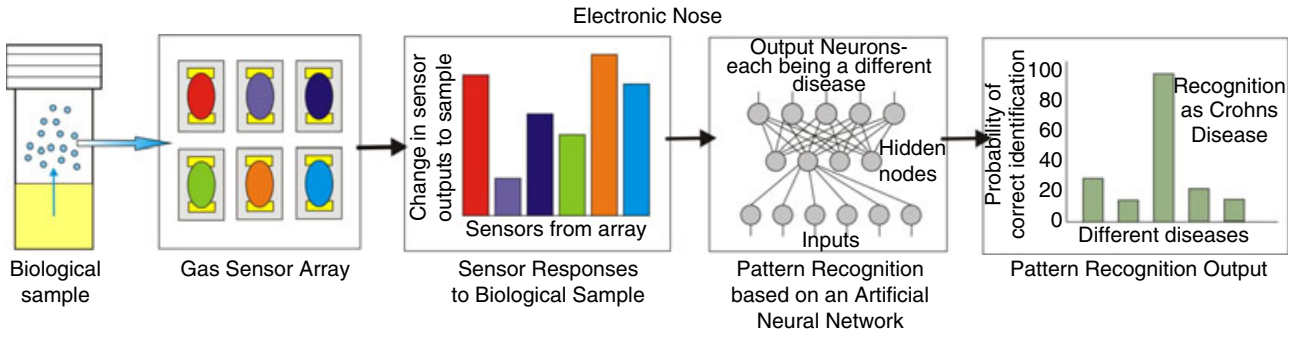

Figure 1 | Modelling of electronic nose based on human olfactory system. Odour molecules from a biological sample are presented to the sensor array and a specific response pattern is generated. This signal is then processed through artificial neural networks to create a pattern recognition output (based on a training set). The output thus provides a probability of the most likely diagnosis based on smell.

include Ion mobility spectrometry (IMS) (Lonestar, Owlstone, Cambridge, UK); BreathSpec (GAS, Dortmund, Germany), GCs employing gas sensors as the detector (zNose; Electronic Sensor Technology, Newbury Park, CA, USA) and optical gas spectrometer instruments (CT3000; Cascade Technologies, Stirling, UK). All of these instruments can be configured to give rapid diagnosis (under 60 s), though IMS generally offers the highest sensitivity of these newer systems. Field asymmetric ion mobility spectroscopy (FAIMS); a type of IMS has shown promise within various medical domains due to its increased sensitivity.[7, 8] FAIMS is able to track the mobility of single ions as it traverses an electric field – making it highly sensitive and thus applicable in detecting minute changes in the make-up of VOCs.

Recently selected ion flow tube mass spectrometry (SIFT-MS) that combines chemical ionisation and mass spectroscopy has been used to detect gas phase volatile compound biomarkers (Voice200; Syft Technologies, Christchurch, New Zealand). It allows rapid quantification of trace volatile compounds even when there is an abundance of atmospheric gases. The specific ion products are detected downstream by quadruple mass spectrometer and an ion-counting system.[9] Table 1 lists the current gas analysis instruments; some of which are already in use within the medical domain, with typical cost per instrument.

Animals specifically canines have been trained to detect malignancies in the breast, bladder and colon by smelling biological fluids.[2–4] However, this process takes a considerable period of training and expense. Mammals also undergo a phenomenon known as olfactory fatigue in which the olfactory bulb becomes saturated with odour molecules rendering it ineffective.[10] Electronic nose instruments are more consistent and reproducible, and although the sensors' sensitivity can drift, they can be re-calibrated.

Gas phase volatile compound biomarker detection in gastroenterology

Gas phase volatile compound biomarkers originate within a biological system and can be assessed using a number of methods. A plausible source of these biomarkers is the human colon.

Health and disease. Fermentation of undigested foods in the colon by its resident bacteria is thought to affect not only colonic health but also influences metabolic health.[11] The anatomical structure of the colon is suited to act as a fermenting chamber with the gaseous molecules emitted having direct effects on colonocytes as well as gut neural and metabolic effects. This complex system referred to as the 'fermentome' can be altered through dietary modification, which will have a direct impact on colonic as well as metabolic health and disease.[12] The gases emitted may play a role in bacterial chemical signalling within the colon, sometimes referred to as quorum sensing[13] but importantly, could also serve as gas phase biomarkers.

Perturbance of the host gut microbiota is known to influence colonic and metabolic health. Assessing resident bacterial populations in the colon requires prolonged culture or expensive genomic sequencing; both are often unsuccessful. Clearly, this is not practical for daily clinical practice. Studies have reported changes in the fermentome produced by patients undergoing

Table 1 | Table of gas analysis instruments describing its advantages and limitations including practical applicability and cost

Technique	Breadth of analysis	Sensitivity	Specificity	Accuracy	Speed	User skill level	Consumable cost per item	Maintenance	Sample cost	Est. Cost (£)
Gas analyzer (e.g. nitric)	Low	Low	Medium	Medium	Real-time	Low	Low	Low	Low	<10k
Electronic nose	Medium	High	Medium	High	Real-time	Low	Low	Low	Low	<40k
Ion mobility spectrometer	Medium	Medium/ High*	Medium/ High	High	Real-time	Medium/ High	Low	Medium	Low	<50k
GC-MS	High	Very high†	Very High	High	Off-line	High	Medium	High	Medium	>150k
PCR-MS	Medium	High	High	High	Real-time	High	Low	Medium	Low	>200k
SIFT-MS	High	High	High	High	Real-time	Medium/ High	Low	Medium	Low	>200k
FAIMS	High	High	High	High	Real-time	High	Low	Medium	Low	<50k
Electronic Nose subgroups										
Metal oxide	Medium	High	Medium	Medium	Real-time	Low	Low	Low	Low	<40k
Optical	Medium	Medium	Medium	High	Real-time	Low	Low	Low	Low	<40k
Polymer	Medium	Medium	Low	Low	Real-time	Low	Low	Low	Low	<40k
GC-Based	High/ Medium	Medium/ High	High	Medium	Real-time	Medium	Medium	Medium	Medium	<40k
Electrochemical	Low	High	High	High	Real-time	Low	Low	Low	Low	<40k
Quartz Crystal	Medium	Medium	Medium	High	Real-time	Medium	Low	Low	Low	<40k

GC-MS, gas chromatography mass spectroscopy; MS, mass spectrometry; SIFT-MS, selected ion flow tube mass spectrometry; FAIMS, field asymmetric ion mobility spectroscopy.

* Depends upon type of IMS technology deployed. Drift tubes are medium, FAIMS high.

† Pre-concentration required.

complete vs. partial bowel cleansing using urine samples. Electronic nose technology was able to distinguish between individuals who had complete compared with partial bowel preparation. Moreover in a subset of individuals, electronic nose was able to identify evolving bacterial re-colonisation over time – offering a practical non-invasive approach to track bacterial dysbiosis following iatrogenic or idiopathic perturbation.[14]

Breath analysis offers an attractive non-invasive option, but a major confounder is trying to detect and quantify tiny amounts of gases/VOCs in the presence of atmospheric gases – the latter often existing in larger quantities and molecular weights. This shortcoming can be circumvented with the use of SIFT-MS technology, which is highly sensitive and able to separate ionised particles even in the presence of other gases present at the time of sampling.[9] Aerosolised particles in breath arise from different sources, and have to contend with metabolites from oral microflora. Additionally inhaled volatile compounds can undergo endogenous degradation, thus altering the ratio between inhaled and exhaled gas concentrations. The latter is important to consider, especially during analysis of acute metabolic conditions.[15] VOC breath analysis (breathomics) does offer the potential to go beyond just diagnostics, but also as biomarkers to monitor oxidative stress and inflammation – e.g. in lung cancer with good results.

Inflammatory bowel disease. The ability to detect gas phase biomarker signatures in the breath of patients with inflammatory bowel disease (IBD) was a significant advance in the 1990s. In a pilot study, alkanes, ethane and pentane were the main chemical groups identified in patients with ulcerative colitis (UC).[16–18] Similar findings were observed using gas chromatography-time-of-flight-mass spectroscopy (GC-TOF-MS). Here, distinction was made between IBD and controls with sensitivities of >90% using six discriminatory VOCs (not specified) within breath. However, sensitivities dropped to 80% when attempting to distinguish active IBD from those in remission.[19] More recently, using different technology such as SIFT-MS, improved distinction using breath was made between Crohn's disease and active UC patients.[20] Systemic pentane production was predominant and is thought to reflect cellular lipid peroxidation – a consequence of mucosal inflammation.

Gut microbiota and altered mucosal permeability have been implicated in the pathogenesis of IBD. The

organisms can ferment nonstarch polysaccharides in the colon producing a fermentation signature. These constitute groups of molecules that can reach the systemic circulation and then urine by passing through an impaired gut mucosal barrier. Using urine samples from 62 patients with Crohn's disease and UC, the electronic nose was able to separate those with IBD and controls and reclassify them with 75% sensitivity and specificity; $p < 0.001$.[21] The instrument could also distinguish between active disease and those in remission. In another study of faecal volatiles using standard thermal desorption (TD) GC-MS, investigators were able to separate those with IBD and controls which included healthy subjects and patients with irritable bowel syndrome.[22] Patients with Crohn's disease had evidence of increased concentrations of ester and derivatives of short-chain fatty acids. Following treatment, these volatile compounds approached levels seen in healthy controls. These studies suggest that the fermentome in IBD is disease-specific when compared to healthy subjects possibly reflecting alteration in bacterial diversity when moving from health to disease. The fermentome signature could also be tracked non-invasively as the disease enters remission (fermentonomics).

Bile acid diarrhoea. In a study of 110 urine samples from patients with bile acid diarrhoea, an electronic nose was able to separate those with bile acid malabsorption from ulcerative colitis and healthy controls with 85% sensitivity.[23] 2-Propanol and acetamide were unique chemicals found in the urine of patients with bile acid diarrhoea and not in controls.

Gastrointestinal cancer. In patients with pelvic cancer using stool samples, the electronic nose detected those that developed severe gastrointestinal-related toxicity following radiotherapy and also importantly, could predict those patients who were more likely to develop severe gut-related toxicity.[24] The sensor response from the e-nose suggested the presence of hydrogen sulphide production which could indicate the presence of hydrogen-producing bacteria, namely firmicutes. It is plausible that the dominance of certain radiation-resistant bacteria, e.g. archaebacteria may determine the risk of toxicity following radiotherapy. In a further pilot series of 47 patients, using urine samples, the electronic nose was able to separate those with colon cancer from healthy controls and those with UC (sensitivity of 87%; $p < 0.001$).[25] Recent studies analysing both breath and faecal material have been used successfully to distinguish between colon cancer and healthy individuals.[26, 27] In the study of breath analysis, conventional GC-MS was utilised with a sensitivity and specificity of 85%. The study assessing faecal volatiles used an electronic nose Cyrano A320 which had a sensitivity and specificity of 92%.

Similarly, assessment of volatile compounds from gastric fluid content has been shown to distinguish gastric cancer from healthy controls using conventional GC-MS as well as SIFT-MS.[28, 29] The gaseous markers identified included mainly alcohols, aldehydes and ketones.

Coeliac disease. selected ion flow tube mass spectrometry technology has been used to separate patients with coeliac disease from healthy controls based on breath volatiles. This was based on the identification of alcohol fermentation products thought to predominate as a result of mal-fermentation of carbohydrates in these patients – no difference was found as only alcohol by-products were sought.[30] When solid phase micro-extraction (SPME) coupled with conventional GC-MS was used in children (aged 6–12 years) with coeliac disease, less volatile compounds were detected in faecal samples compared with healthy controls consisting of patients' siblings or treated coeliac disease. Corresponding faecal microbiota analysed by PCR-denaturing gradient gel electrophoresis in children with coeliac disease showed reduced diversity, especially for the *Lactobacilli* and *Bifidobacterium* species.[31] The latter is thought to influence host immunity which may contribute in part to activation of the innate immune system.

Liver disease. In liver disease, detection of gas phase biomarkers within breath has been used to separate those with hepatocellular carcinoma[32, 33] and non-alcohol fatty liver disease.[34] Liver disease results in metabolic derangement with production of endogenous compounds which concentrate in the blood. Some of these are volatiles which can be measured in urine and breath. The imbalance of gut microbiota noted in liver cirrhosis[35] could account for the differential colonic fermentation profiles which can be detected as volatile compounds. Dimethylsulphide (DMS) is a volatile sulphur compound that is thought to be responsible for the odour of foetor hepaticus.[36] DMS is formed by anaerobic bacterial breakdown of sulphur-containing amino acids. It is a stable molecule that is present in biological tissues including blood, urine and breath.[37]

In a study using SPME coupled with conventional GC, increases in ester compounds were noted in faeces

of those with non-alcohol fatty liver disease (NAFLD); about a third of the volatile compounds were also detected in healthy subjects. The remaining two-thirds of the volatile variation in NAFLD corresponded with an increase in *Lachnospiraceae and Lactobacilli* genus.[34]

Gut infections. The need for rapid diagnosis of infections compared with prolonged microbiological culture has resulted in the exploration of volatile organic compounds to provide a rapid diagnosis. Using SPME GC-MS on faecal samples from patients with *C. difficile*-associated diarrhoea, an abundance of furan metabolites with corresponding reduction in methylindole was found. The latter is thought to be due to the disruption of nosocomial *E. coli* resulting from broad-spectrum antibiotic usage. Long-chain fatty acids such as ethyl dodecanoate were noted in those with rotavirus infections whilst the presence of ammonia without ethyl dodecanoate suggested other enteric viruses and the absence of hydrocarbons and terpenes indicated *Campylobacter* sp. infection.[38] In healthy individuals, almost half of volatile compounds from faeces (mainly derivatives of short-chain fatty acids) are shared between individuals and are relatively stable over a period of at least 2 weeks – with little alteration as a result of diet.[39]

In pilot studies, patients with *Helicobacter pylori* (HP) infection could be separated from controls by measuring breath volatiles.[40] Discrimination of HP from other bacterial gastro-oesophageal isolates was also possible using an electronic nose.[41] The predominant volatile compounds were isobutene, 2-butanone and ethyl acetate. Others have reported detection of hydrogen cyanide and hydrogen nitrate in those with HP-associated gastritis compared with controls.[42]

Gas phase biomarker detection in other diseases

Lung disease. Previous research since the 1990s, utilising GC-MS, has shown detectable gas phase markers in exhaled breath in different disease states. The use of the electronic nose in diagnosing respiratory disease was therefore an obvious starting point.[43–55] Several studies have examined the use of an electronic nose in respiratory infection, both in a clinical setting and in *in vitro* assessments. *In vitro* studies show that the odour contained in the headspace of different microbial cultures can be detected by the electronic nose, and can be differentiated from controls.[56–61] When bacterial culture is taken as the gold standard, electronic nose has a sensitivity and specificity of over 90% with potential for rapid, organism-specific diagnosis.

The electronic nose has also been shown to have a predictive value of over 90% in differentiating patients with obstructive airways disease[52–55] from those with lung cancer.[49, 62] Thus, it could be used as a screening tool in high-risk groups. There is a growing interest in the detection of pulmonary tuberculosis in breath using electronic nose-based technology with reported sensitivities and specificities of 71% and 72% respectively.[63–65]

Other cancers. The expansion of the electronic nose to detect cancers in other organs using breath volatiles is growing with evidence that the electronic nose can differentiate between cancer and normal cell lines derived from prostate and ovaries and also from brain and skin cancer tissues.[66–69] In a pilot study, bladder cancer could also be correctly re-classified with >90% accuracy using GC and a single metal oxide detector from urine samples.[70]

Diabetes. The dysregulation of lipids and glucose with ketone formation is the premise for VOC detection in patients with diabetes. Aerosolised glucose and aromatic compounds like isoprene have been shown to distinguish between type 2 diabetes and healthy controls with a sensitivity of 90% and specificity of 92%.[71] Interestingly, gas volatile marker correlation with blood was better in type 1 compared with type 2 diabetes.[72] Nevertheless, gas phase markers as a screening tool for detection of metabolic disorders including hyperglycaemia and hyperlipidaemia offer great potential, especially in primary care.

Halitosis. Halitosis can be attributed to intraoral and extraoral causes. Determination of the predominant compound can provide clues as to its aetiology. Acetones and other ketones are linked with diabetes mellitus and weight reduction, dimethylsulphide, C2-C5 aliphatic and isovaleric acid with liver diseases, whilst hydrogen sulphide and methyl mercaptan with oral malodour. Patients with oropharyngeal carcinoma are thought to produce a particular breath odour due to branched chain organic acids (C_2–C_8 compounds), which can be detected by GC-MS.[42]

Not surprisingly, VOCs measured by GC-MS from oral microbial cultures show a range of volatile compounds within the headspace. Specifically, anaerobic species producing hydrogen sulphide, methyl mercaptan and indoles give rise to malodour. Interestingly, volatiles

produced from the tongue biofilm can vary according to the pH of the growth media it is maintained. For example, increasing the pH from 6 to 8 enables detection of Dimethyl di and trisulphide and other volatile sulphur compounds.[73] The latter compounds have also been previously reported in faeces.[74, 75] Of note is the overlap of chemicals reported in breath of patients with liver disease.

Sniffing the future – electronic hand-helds

It is our belief that the ultimate gas phase biomarker detection will be achieved through closely mimicking the biological olfactory system. This can be achieved by combining large numbers of diverse micro-sensor arrays. The human nose has ~350–400 different types of functional olfactory receptors with each containing millions of neurones. Each sample will be offered to the sensors in a carefully controlled system mimicking the human nose which filters, controls the temperature and flow rate to the olfactory bulb.[76–78] The olfactory system also has a 'nasal chromatograph' that aids in separating chemical components before contact with olfactory receptors – coupled with advanced biological data processing of the complex spatial and temporal responses.[76–80] This combined approach could be the future basis of new cutting-edge instruments.

We are already seeing the first portable/hand-held medical diagnostic devices near to commercialisation for gastroenterological diseases, including tuberculosis

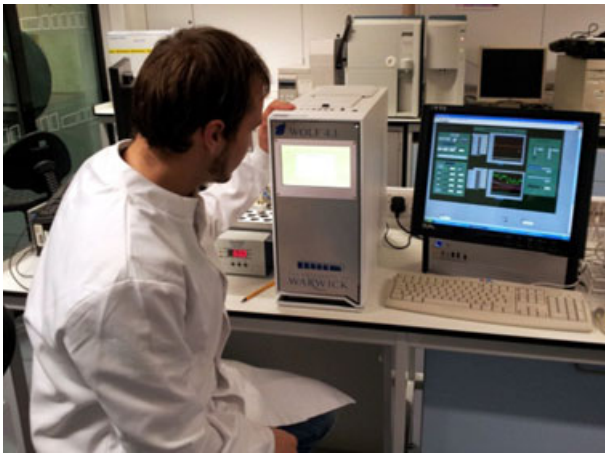

Figure 2 | A new generation electronic nose – WOLF system (Warwick OLFaction electronic nose; Current version WOLF 4.1). This unit houses a combination of modern nano-material sensors with specialised sample capture, data extraction and analytical software.

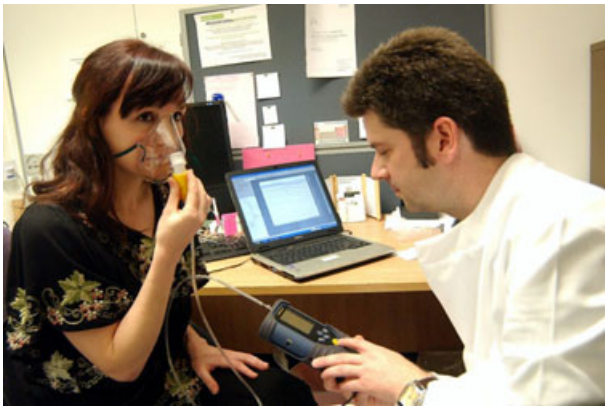

Figure 3 | Example of a smaller, hand-held electronic nose device version; 2.1 prototype in development for breath capture of VOCs.

(Aeonose; The Electronic Nose Company, Zutphen, Netherlands), *Clostridium difficile* (Odoreader; University of the West of England, Bristol, UK) and bacterial overgrowth (Gastrocheck; Bedfont Scientific, Maidstone, UK). Recent advances in the field of nanomaterials for chemical sensing such as graphene, carbon nanotubes and nanostructured materials[81–83] will expand the field of devices available (Figures 2 and 3).

There are many efforts in developing 'nose-on-a-chip' solutions, micro-electronic devices that combine the sensor and sensing material, electronic interface and the decision-making process onto a silicon chip that could eventually be integrated into a mobile/portable device.[84–86] An example is the latest Samsung S4 mobile phone which already has an integrated temperature and humidity sensor produced by Sensirion (AG, Wald, Switzerland) and is only 2 mm × 2 mm in size (http://www.chipworks.com/en/technical-competitive-analysis/resources/blog/inside-the-samsung-galaxy-s4/). Such efforts demonstrate the desire to make instruments that closely mimic the biological olfactory system, which, we believe, will ultimately allow sniffing technology at point of care.

CONCLUSIONS

Gas phase volatile compound biomarkers offer the potential for future diagnostics in gastroenterology/medicine. It provides a distinct advantage but equally requires specialised sensitive instrumentation to capture and analyse samples. The electronic nose stands up to the challenge as evidence mounts in favour of its support. The combined efforts of engineers, chemists and clinicians together with advancing technology will enable smaller

hand-held VOC sensing devices that can be used in point of care, thus bringing closer the reality of non-invasive diagnostic medicine.

AUTHORSHIP

Guarantor of the article: R. P. Arasaradnam.
Author contributions: RA, JC, CH and CN contributed equally to the writing of the article. RA, JC and CN performed the final editing. RA, JC and CH performed detailed literature review. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

Declaration of personal and funding interests: None.

REFERENCES

1. Pauling L, Robinson A, Teranishi R. Quantitative analysis of urine vapour and breath by gas liquid partition chromatography. *Proc Natl Acad Sci USA* 1971; **68**: 2374–6.
2. Willis CM, Church SM, Guest CM, *et al.* Olfactory detection of human bladder cancer by dogs: proof of principle study. *BMJ* 2004; **329**: 712–5.
3. McCulloch M, Jezierski T, Broffman M, *et al.* Diagnostic accuracy of canine scent detection in early-and late-stage lung and breast cancers. *Integr Cancer Ther* 2006; **5**: 30–9.
4. Sonoda H, Kohnoe S, Yamazato T, *et al.* Colorectal cancer screening with odour material by canine scent detection. *Gut* 2011; **60**: 814–9.
5. Wilson AD, Baietto M. Review: advances in electronic-nose technologies developed for biomedical applications. *Sensors* 2011; **1**: 1105–76.
6. Dutta R, Morgan D, Baker N, *et al.* Identification of *Staphylococcus aureus* infections in hospital environment: electronic nose based approach. *Sens Act B Chem* 2005; **109**: 355–62.
7. Arshak K, Moore E, Lyons GM, *et al.* A review of gas sensors employed in electronic nose applications. *Sens Rev* 2004; **24**: 181–98.
8. Guevremont R, Purves RW. Atmospheric pressure ion focusing in a high-field asymmetric waveform ion mobility spectrometer. *Rev Sci Instrum* 1999; **70**: 1370.
9. Smith D, Spanel P. Selected Ion Flow Technology (SIFT-MS) for online gas analysis. *Mass Spectrom Rev* 2005; **24**: 661–700.
10. Arasaradnam RP, Nwokolo CU, Bardhan KD, *et al.* Electronic nose versus canine nose: clash of the titans. *Gut* 2011; **60**: 1768.
11. Arasaradnam RP, Pharaoh MW, Williams GJ, *et al.* Colonic fermentation–more than meets the nose. *Med Hypotheses* 2009; **73**: 753–6.
12. Arasaradnam RP, Quraishi N, Kyrou I, *et al.* Insights into 'fermentonomics': evaluation of volatile organic compounds (VOCs) in human disease using an electronic 'e-nose'. *J Med Eng Technol* 2011; **35**: 87–91.
13. Miller M, Bassler B. Quorum sensing in bacteria. *Annu Rev Microbiol* 2001; **55**: 165–99.
14. Arasaradnam RP, Ouaret N, Thomas MG, *et al.* Evaluation of gut bacterial populations using an electronic e-nose and field asymmetric ion mobility spectrometry: further insights into 'fermentonomics'. *J Med Eng Technol* 2012; **36**: 333–7.
15. Boots AW, van Berkel JJ, Dallinga J, *et al.* The versatile use of exhaled volatile organic compounds in human health and disease. *J Breath Res* 2012; **6**: 027108.
16. Kokoszka J, Nelson RL, Swedler WI, *et al.* Determination of inflammatory bowel disease activity by breath pentane analysis. *Dis Colon Rectum* 1993; **36**: 597–601.
17. Sedgh S, Keshavarzian A, Klamut M, *et al.* Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 1994; **89**: 2217–21.
18. Pelli MA, Trovarelli G, Capodicasa E, *et al.* Breath alkanes determination in ulcerative colitis and Crohn's disease. *Dis Colon Rectum* 1999; **42**: 71–6.
19. Bodelier A, Smolinska A, Dallinga J, *et al.* Volatile organic compound in breath as a new test for Crohn's disease activity. *United Eur Gastroenterol J* 2013; **1**: A1.
20. Dryahina K, Spanel P, Pospisilova V, *et al.* Quantification of pentane in exhaled breath, a potential biomarker of bowel disease, using selected ion flow mass spectrometry. *Rapid Commun Mass Spectrom* 2013; **27**: 1983–92.
21. Arasaradnam RP, Ouaret N, Thomas MG, *et al.* A novel tool for non-invasive diagnosis and tracking of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 999–1003.
22. Walton C, Fowler DP, Turner C, *et al.* Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. *Inflamm Bowel Dis* 2013; **19**: 2069–78.
23. Covington JA, Westenbrink EW, Ouaret N, *et al.* Application of a novel tool for diagnosing bile acid diarrhoea. *Sensors (Basel)* 2013; **13**: 11899–912.
24. Covington JA, Wedlake L, Andreyev J, *et al.* The detection of patients at risk of gastrointestinal toxicity during pelvic radiotherapy by electronic nose and FAIMS: a pilot study. *Sensors (Basel)* 2012; **12**: 13002–18.
25. Covington J, Ryan-Fisher C, Westinbrink E, *et al.* Towards novel non invasive detection of colon cancer using electronic nose and FAIMS. *Gut* 2013; **62**: A218–9.
26. Altamore DF, Di Lena M, Porcelli F. Exhaled volatile organic compounds detect patients with colon cancer. *Br J Surg* 2013; **100**: 144–50.
27. de Meij T, Larbi I, van der Schee MP, *et al.* Electronic nose can discriminate colorectal carcinoma and advanced adenomas by faecal volatile markers; proof of principle study. *Int J Cancer* 2013; **134**: 1132–8.
28. Buszewski B, Ulanowska A, Ligot T, *et al.* Identification of volatile organic compounds secreted from cancer tissues and bacterial cultures. *J Chromatogr B* 2008; **868**: 88–94.
29. Kumar S, Huang J, Cushmir J, *et al.* Selected ion flow tube-MS analysis of headspace vapour from gastric content for the diagnosis of gastro-oesophageal cancer. *Anal Chem* 2012; **84**: 9550–7.
30. Hryniuk A, Ross B. A preliminary investigation of exhaled breath from patients with coeliac disease using selected ion flow tube mass spectrometry. *J Gastrointestin Liver Dis* 2010; **1**: 15–20.
31. Di Cagno R, Rizello C, Gagliardi F, *et al.* Different faecal microbiota and volatile organic compounds in treated and untreated children wtih coeliac

disease. *Appl Environ Microbiol* 2009; **75**: 3963–71.
32. Ilan Y. Review article: the assessment of liver function using breath tests. *Aliment Pharmacol Ther* 2007; **26**: 1293–302.
33. Qin T, Liu H, Song Q, *et al.* The screening of volatile markers for hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2247–53.
34. Raman M, Ahmed I, Gillevet PM, *et al.* Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868–75.
35. Zhang W, Gu Y, Chen Y, *et al.* Intestinal flora imbalance results in altered bacterial translocation and liver function in rats with experimental cirrhosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1481–6.
36. Tangerman A, Meuwese-Arends M, Jansen J. Cause and composition of foetor hepaticus. *The Lancet* 1994; **343**: 483.
37. Van den Velde S, Nevens F, Van Hee P, *et al.* GC-Ms analysis of breath odour compounds in liver patients. *J Chromatogr B* 2008; **875**: 344–8.
38. Probert C, Jones P, Ratcliffe N. A novel methods for rapidly diagnosing the cause for diarrhoea. *Gut* 2004; **1**: 58–61.
39. Garner C, Smith S, Costello B, *et al.* Volatile organic compounds from faeces and heir potential for diagnosis of gastrointestinal disease. *FASEB J* 2007; **21**: 1675–88.
40. Lechner M, Karlseder A, Niederseer D, *et al.* H Pylori infection increases levles of exhaled nitrate. *Helicobacter* 2005; **10**: 385–90.
41. Pavlou AK, Magan N, Sharp D, *et al.* An intelligent rapdi odour detection model in discrimination of Helicobacter pylori infection and other gastroesophageal isolates in vitro. *Biosens Bioelectron* 2000; **15**: 333–42.
42. Miekisch W, Schubert JK, Noeldge-Schomburg GF. Diagnositoc potential of breath analysis – focus on volatile organic compounds. *Clin Chim Acta* 2004; **347**: 25–39.
43. Corradi M, Majori M, Cacciani GC, *et al.* Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. *Thorax* 1999; **54**: 572–5.
44. Montuschi P, Corradi M, Ciabattoni G, *et al.* Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999; **160**: 216–20.
45. Olopade CO, Christon JA, Zakkar M, *et al.* Exhaled pentane and nitric oxide levels in patients with obstructive sleep apnea. *Chest* 1997; **111**: 1500–4.
46. Olopade CO, Zakkar M, Swedler WI, *et al.* Exhaled pentane levels in acute asthma. *Chest* 1997; **111**: 862–5.
47. Paredi P, Kharitonov SA, Barnes PJ. Elevation of exhaled ethane concentration in asthma. *Am J Respir Crit Care Med* 2000; **162**: 1450–4.
48. Thaler ER, Kennedy DW, Hanson CW. Medical applications of electronic nose technology: review of current status. *Am J Rhinol* 2001; **15**: 291–5.
49. Dragonieri S, Annema JT, Schot R, *et al.* An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. *Lung Cancer* 2009; **64**: 166–70.
50. Lazar Z, Fens N, van der Maten J, *et al.* Electronic nose breathprints are independent of acute changes in airway caliber in asthma. *Sensors (Basel)* 2010; **10**: 9127–38.
51. Montuschi P, Santonico M, Mondino C, *et al.* Diagnostic performance of an electronic nose, fractional exhaled nitric oxide, and lung function testing in asthma. *Chest* 2010; **137**: 790–6.
52. Fens N, de Nijs SB, Peters S, *et al.* Exhaled air molecular profiling in relation to inflammatory subtype and activity in COPD. *Eur Respir J* 2011; **38**: 1301–9.
53. Fens N, Roldaan AC, van der Schee MP, *et al.* External validation of exhaled breath profiling using an electronic nose in the discrimination of asthma with fixed airways obstruction and chronic obstructive pulmonary disease. *Clin Exp Allergy* 2011; **41**: 1371–8.
54. Hattesohl AD, Jorres RA, Dressel H, *et al.* Discrimination between COPD patients with and without alpha 1-antitrypsin deficiency using an electronic nose. *Respirology* 2011; **16**: 1258–64.
55. Bofan M, Mores N, Baron M, *et al.* Within-day and between-day repeatability of measurements with an electronic nose in patients with COPD. *J Breath Res* 2013; **7**: 017103.
56. Lai SY, Deffenderfer OF, Hanson W, *et al.* Identification of upper respiratory bacterial pathogens with the electronic nose. *Laryngoscope* 2002; **112**: 975–9.
57. Hockstein NG, Thaler ER, Torigian D, *et al.* Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. *Laryngoscope* 2004; **114**: 1701–5.
58. Pavlou AK, Magan N, Jones JM, *et al.* Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. *Biosens Bioelectron* 2004; **20**: 538–44.
59. Hockstein NG, Thaler ER, Lin Y, *et al.* Correlation of pneumonia score with electronic nose signature: a prospective study. *Ann Otol Rhinol Laryngol* 2005; **114**: 504–8.
60. Humphreys L, Orme RM, Moore P, *et al.* Electronic nose analysis of bronchoalveolar lavage fluid. *Eur J Clin Invest* 2011; **41**: 52–8.
61. de Heer K, van der Schee MP, Zwinderman K, *et al.* Electronic nose technology for detection of invasive pulmonary aspergillosis in prolonged chemotherapy-induced neutropenia: a proof-of-principle study. *J Clin Microbiol* 2013; **51**: 1490–5.
62. Machado RF, Laskowski D, Deffenderfer O, *et al.* Detection of lung cancer by sensor array analyses of exhaled breath. *Am J Respir Crit Care Med* 2005; **171**: 1286–91.
63. Fend R, Kolk AH, Bessant C, *et al.* Prospects for clinical application of electronic-nose technology to early detection of Mycobacterium tuberculosis in culture and sputum. *J Clin Microbiol* 2006; **44**: 2039–45.
64. Philips M, Basa-Dalay V, Blais J, *et al.* Point of care breath testing for biomarkers of active tuberculosis. *Tuberculosis* 2012; **92**: 314–22.
65. Banday KM, Pasikanti K, Chan ECY, *et al.* Use of urine volatile organic compounds to discriminate tuberculosis patients from healthy subjects. *Anal Chem* 2011; **83**: 5526–34.
66. Horvath G, Chilo J, Lindblad T. Different volatile signals emitted by human ovarian carcinoma and healthy tissue. *Future Oncol* 2010; **6**: 1043–9.
67. Roine A, Tolvanen M, Sipilainen M, *et al.* Detection of smell print differences between nonmalignant and malignant prostate cells with an electronic nose. *Future Oncol* 2012; **8**: 1157–65.
68. Kateb B, Tyan MA, Homer ML, *et al.* Sniffing out cancer using the JPL electronic nose: a pilot study of a novel approach to detection and differentiation of brain cancer. *Neuroimage* 2009; **47**: T5–9.
69. D'Amico A, Bono R, Pennazza G, *et al.* Identification of melanoma with gas sensor array. *Skin Res Technol* 2008; **14**: 226–36.
70. Khalid T, White P, Costello B, *et al.* A Pilot study combining a GC-Sensor device with a statistical model for the identificatino of bladder cancer from urine headspace. *PLoS ONE* 2013; **8**: e696902.
71. Minh TDC, Blake DR, Galassetti PR. The clinical potential of exhaled breath

analysis for diabetes mellitus. *Diabetes Res Clin Pract* 2012; **97**: 195–205.
72. Greiter MB, Keck L, Siegmund T, *et al.* Differences in exhaled gas profiles between patients with type 2 diabetes and healthy controls. *Diabetes Technol Ther* 2010; **12**: 455–63.
73. Khalid T, Saad S, Greenman J, *et al.* Volatiles from oral anaerobes confounding breath biomarker discovery. *J Breath Res* 2013; **7**: 017114.
74. Suarez FL, Springfield J, Levitt M. Identification of gases responsible for the odour of human flatus and evaluation of a device purported to reduce this odour. *Gut* 1998; **43**: 100–4.
75. Moore J, Jessop LD, Osborne D. Gas Chromatogaphic and mass-spectrometric analysis of the odour of human faeces. *Gastroenterology* 1987; **93**: 1321–9.
76. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 1991; **65**: 175–87.
77. Glusman G, Yanai I, Rubin I, *et al.* The complete human olfactory subgenome. *Genome Res* 2001; **11**: 685–702.
78. Mainland J, Keller A, Li YR, *et al.* The missense of smell: functional variability in the human odorant receptor repertoire. *Nat Neurosci* 2013; **17**: 114–20.
79. Kent PF, Mozell MM, Youngentob SL, *et al.* Mucosal activity patterns as a basis for olfactory discrimination: comparing behavior and optical recordings. *Brain Res* 2003; **15**: 1–12.
80. Mozell MM, Sheehe PR, Hornung DH, *et al.* "Imposed" and "inherent" mucosal activity pattern. Theircomposite representation of olfactory stimuli. *J Gen Physiol* 1987; **90**: 625–50.
81. Fama DH, Palaniappana AL, Toka ALY, *et al.* A review on technological aspects influencing commercialization of carbon nanotube sensors. *Sens Act B Chem* 2011; **1**: 1–7.
82. Pandey PA, Wilson NS, Covington JA. Pd-doped reduced graphene oxide sensing films for H2 detection. *Sens Act B Chem* 2013; **183**: 478–87.
83. Llobet E. Gas sensors using carbon nanomaterials: a review. *Sens Act B* 2013; **179**: 32–45.
84. Che Harun FK, Taylor JE, Covington JA, *et al.* An electronic nose employing dual-channel odour separation columns with large chemosensor arrays for advanced odour discrimination. *Sens Act B Chem* 2009; **1**: 134–40.
85. Tang KT, Chiu SW, Chang MF, *et al.* A low-power electronic nose signal-processing chip for a portable artificial olfaction system. *IEEE Trans Biomed Circuits Syst* 2011; **5**: 380–90.
86. MIT Technology Review. Available at: http://www.technologyreview.com/view/427935/a-smart-phone-that-can-sniff-out-sickness/. Accessed January 2, 2014.