

Proceeding

Recent Applications of Electronic-Nose Technologies for the Noninvasive Early Diagnosis of Gastrointestinal Diseases [†]

Alphus Dan Wilson

Southern Hardwoods Laboratory, Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, 432 Stoneville Road, Stoneville, MS 38776, USA; dwilson02@fs.fed.us; Tel.: +1-662-686-3180

[†] Presented at the 4th International Electronic Conference on Sensors and Applications (ECSA 2017), 15–30 November 2017; Available online: <https://sciforum.net/conference/ecsa-4>.

Published: 14 November 2017

Abstract: Conventional methods for diagnosing gastrointestinal (GI) diseases have involved analysis of headspace volatile organic compounds (VOCs) present in the breath, urine, or fecal samples of patients. Most previous diagnostic testing methods have utilized purely metabolomic approaches to analyze VOCs with analytical instruments such as gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) metabolomics, selected ion flow tube-mass spectrometry (SIFT-MS), proton transfer reaction-mass spectrometry (PTR-MS), and field asymmetric ion mobility spectrometry (FAIMS). These sophisticated and expensive methods usually involve the use of large immobile (non-portable) benchtop instruments, requiring extensive data manipulations and analyses along with advanced modeling procedures to achieve diagnostic interpretations of complex chemical data. Colonoscopies and biopsies are more invasive and discourage patient-participation in prophylactic GI-disease screenings. The more recent availability of portable electronic nose gas-sensing devices, developed with the aim of simplifying disease diagnoses by analysis of headspace VOC mixtures collectively using multi-sensor arrays, allow the production of disease-specific aroma signatures (VOC profiles) based on detection of precise complex mixtures of disease biomarker metabolites. Electronic-nose (e-nose) devices provide very fast results, are easy to operate, and are more readily applicable to clinical practice. This paper summarizes some very recent e-nose technologies being developed and tested for GI-disease diagnostic applications, including ones with dual-technology and multi-technology sensor arrays for both pattern recognition and identification of key-metabolite chemical species. In addition, novel portable electronic devices, developed with new operational mechanisms and sensor types, are described which offer possibilities of providing new means of diagnosing GI-tract diseases.

Keywords: colitis; disease biomarkers; early noninvasive diagnoses, electronic aroma detection; e-nose devices; healthcare applications; metabolite signatures; volatile organic compounds

1. Introduction

Disease diagnostics research recently has undergone significant changes through a series of progressive transformations as new disease-detection technologies have been introduced with greater capabilities of chemical discrimination. Previous older and cumbersome methods used in the detection of gastrointestinal-tract (GI-tract) diseases have involved predominantly colonoscopies, tissue biopsies, and microbial-culture tests to assess causes of disease [1]. These often invasive, time-consuming, and expensive methods are not appropriate for large-scale disease-screening purposes and consequently have been replaced mostly by more cost-effective sophisticated chemical-detection tests involving measures of changes in VOC metabolites (metabolomics), produced as a result of

disease processes, which alter normal physiological and metabolic pathways occurring in affected tissues of the GI-tract. Metabolomic-type diagnostic approaches assess changes in the types and quantities of specific volatile organic compound (VOC) metabolites produced as a result of disease processes (pathogenesis). Purely metabolomic methods utilize complex instruments such as gas chromatography-mass spectroscopy (GC-MS), nuclear magnetic resonance (NMR) metabolomics, selected ion flow tube-mass spectrometry (SIFT-MS), proton transfer reaction-mass spectrometry (PTR-MS), and field asymmetric ion mobility spectroscopy (FAIMS) [2–6]. By contrast, electronic-nose (e-nose) instruments allow recognition of complex mixtures of disease biomarkers without identifying individual chemical species. This simpler approach ultimately promises to greatly accelerate the noninvasive early diagnosis of GI-tract diseases, allowing earlier more effective treatments, more rapid patient recovery, and shorter less-expensive stays for hospital care [7].

2. Mechanisms and Theory of Gastrointestinal-Disease Detection

The methods used for the detection of gastrointestinal diseases largely depend on the types and mechanisms of individual GI-diseases, the chemical classes of VOC-metabolites released from disease tissues, and the mobility of disease-associated metabolites in the body, including how and where these metabolites are translocated and distributed to other organs and excretory systems (pulmonary, urinary, and gastrointestinal) where these materials are released at various rates from the body.

A variety of VOC gas-sampling methods have been utilized to diagnose various types of GI-tract diseases, including human breath, urine, and fecal (stool) sample analyses. The many types and quantities of headspace VOCs released from fecal samples reflect the overall metabolic state of an individual which may be affected by a wide variety of factors that alter intestinal metabolism including diet, medication and drug use, ingestion of probiotics and antibiotics, types of gastrointestinal resident microbes (GIRMs) present which comprise the GI-microbiome, and presence of disease states in the body [8,9]. Consequently, alterations in metabolism caused by GI-tract infections, inflammation, and related diseased states can result in significant changes in GIRM-composition, altering the complex mixture of VOCs gases released in fecal samples. Modern gas-sensing devices take advantage of observed differences in VOC metabolite signatures (profiles) in diseased patients, different from those of normal healthy individuals, to diagnose many different types of GI-tract diseases. In addition, the detection of specific, unique VOCs known as chemical disease biomarkers, may provide further confirmation of specific disease diagnoses determined from unique fecal VOC smellprint patterns.

The efficacy of utilizing analyses of VOC profiles of diseased tissues for clinical diagnostics has been well established and thoroughly proven effective by numerous studies [10–13]. For GI diseases, analysis of VOCs emitted from fecal samples have shown significant changes in VOC profiles that have helped in disease etiology and identification of disease biomarkers associated with specific diseases [8]. Changes in VOC profiles of the GI-tract, resulting from alterations in host physiology due to disease processes (pathogenesis), also often cause changes in gut microbiota composition due to changes in GI-growth conditions for microbes in diseased patients compared with healthy controls. Also, pathogenic agents of biotic diseases normally produce unique types of metabolites that often change the VOC composition of GI-gas mixtures released from fecal samples extracted from a diseased gut. Analysis of VOC profiles provide clearer understanding of the mechanisms (pathophysiology) and origin of disease resulting from changes in host metabolic pathways [9]. Exhaled breath VOCs, associated with irritable bowel disease (IBD), probably originate from the systemic response and result from gases in the gut that diffuse into the bloodstream and are released into lung alveoli [14]. Certain VOCs that are highly associated and correlated with the presence of specific diseases may serve as effective chemical biomarkers of disease.

2.1. Biomarker Metabolites

The detection of disease-specific biomarker VOCs, either individually using chemical analysis methods or collectively (in complex headspace gas mixtures) using various e-nose technologies, has provided powerful and highly complementary methods and tools for effective disease diagnoses [14].

For example, significant differences in VOC-metabolite patterns derived from GC-MS analyses of headspace gases from stool samples indicated that the four different main causes of infectious diarrhea in hospitals could be discriminated based on unique biomarkers associated with different infectious etiologic agents [15]. Infectious diarrhea caused by: 1) *Clostridium difficile* produced furan biomarkers (without indole functional groups), 2) rotaviruses induced the production of an ethyl dodecanoate (ED) biomarker, while 3) other enteric viruses produced ammonia without the ED-biomarker, and 4) *Campylobacter* species failed to produce terpene and simple hydrocarbons.

Two studies have shown that detection of three specific exhaled VOCs (including 1-octene, 1-decene and (E)-2-nonene) found in breathprints of pediatric patients could be used to distinguish between individuals with IBD and healthy controls [16,17].

2.2. Metabolomic Disease-Detection Approaches

Metabolomic approaches to disease detection attempt to detect changes in the types and quantities of specific volatile organic metabolites (VOMs) produced as a consequence of disease states within the body. These methods are usually expensive and require extensive knowledge and skills in the use of sophisticated chemical instruments as well as complicated chemical-modeling methods and statistics software. GC-MS analyses of fecal samples from 140 patients with chronic irritable bowel syndrome (IBS) revealed 240 VOMs of which 44 key compounds were used to discriminate between individuals with diarrhea-predominant, Crohn's disease, and ulcerative colitis forms using univariate statistical analysis [8]. A similar study of inflammatory bowel disease (IBD) using partial least-squares-discriminate analysis of GC-MS data showed clear separation of patients with Crohn's disease from healthy controls based on the greater abundance of four specific VOM-biomarkers, and less abundance of four additional VOM biomarker chemical classes [18]. Three unique biomarkers (1-octene, 1-decene, and (E)-2-nonene) from unique breathprints in children with IBD were determined by metabolomic analysis of breath VOCs using linear discriminant and principle component analyses with SIFT-MS data [16].

VOC profiling using portable field asymmetric ion mobility spectroscopy (FAIMS) technology recently has shown to have significant advantages over conventional SIFT-MS chemical analyses. FAIMS technology is available as a small, portable point-of-care breath-analysis device that uses air as the carrier gas, allowing real-time separation of VOC profiles. Compared with SIFT-MS, FAIMS operates at a fraction of the cost (10-20%) of SIFT-MS, although SIFT-MS outperforms the diagnostic power of SIFT-MS [19]. Detection of colorectal cancer (CRC) recently was achieved by FAIMS-analysis of headspace urinary VOC signatures using Fisher discriminant analysis [20]. FAIMS also was used to differentiate diagnoses of patients with coeliac disease (CD) from IBD by sparse logistic regression analysis of headspace urinary VOCs and the discovery of a single biomarker (1,3,5,7-cyclooctatetraene) by GC-MS in CD-urine VOCs that was absent in urine from IBD patients [21].

3. Gastrointestinal Disease Types and e-Nose Instruments for Detection

The most prevalent and important diseases of the GI-tract detected with e-nose technologies include colorectal cancer (CRC), inflammatory bowel disease (IBD) including Crohn's Disease (CD) and ulcerative colitis (UC), irritable bowel syndrome (IBS), infectious diarrhea (ID), celiac disease, necrotizing enterocolitis, and cholera [10]. All of these diseases may be diagnosed based on specific abnormal VOCs, released as a consequence of disease, that produce unique sensor output patterns from the e-nose sensor array in response to complex VOC mixtures (present in sample headspace), identified using pattern-recognition algorithms and disease-specific reference libraries.

3.1. Electronic-Nose Instruments for GI-Disease Detection

A wide range of electronic-nose technologies with different operational mechanisms are commercially available for clinical disease diagnostic applications. The most commonly used e-nose

types include conducting polymer (CP), metal oxide semiconductor (MOS), polymer carbon black composite (PCBC), quartz crystal microbalance (QCM), and surface acoustic wave (SAW) devices [6].

3.2. Examples of Recent e-Nose GI-Disease Detection Applications

New experimental e-nose devices are being developed each year with new detection methods and sensor types. Some of the most recent e-nose devices used for detection of GI-tract diseases are summarized in Table 1.

Table 1. Recent applications of electronic-nose technologies for the noninvasive early diagnosis of gastrointestinal diseases.

Disease ¹	Location	Sample	N	E-nose model	Sensor type / no.	References
BAD	Bowel	Urine	110	Fox 4000	MOS 18	[22]
CRC	Colon	Breath	26	Experimental	GNP 14	[23]
	Colon	Fecal	157	Cyranose 320	PCBC 32	[3]
CRC/IBD	Colon	Urine	92	WOLF	EC 8, NDIR 2, PID 1	[1]
IBD	Colon	Fecal	83	Cyranose 320	PCBC 32	[24]
IBS	Colon	Fecal	182	Experimental	GC-MOS 1	[25]
ID	Colon	Fecal	100	Experimental	GC-MOS 1	[26]
LOS	Systemic	Fecal	76	Cyranose 320	PCBC 32	[27]
NEC	Colon	Fecal	27	Cyranose 320	PCBC 32	[28]

¹ Disease abbreviations: BAD = Bile acid diarrhea; CRC = Colorectal cancer; IBD = Inflammatory bowel disease; IBS = Irritable bowel syndrome; ID = Infectious diarrhea; LOS = Late-onset sepsis; NEC = Necrotizing enterocolitis.

Portable gas-sensing e-nose devices will no doubt gain increasing acceptance for routine clinical use as methods and procedures are refined and standardized by worldwide use and more extensive clinical testing. World conferences on e-nose clinical uses could facilitate the standardization process.

4. Novel Experimental Electronic-Nose Devices

The development of novel electronic-nose devices offers new potential tools for clinical disease diagnosis. Some e-nose devices have new operational mechanisms and sensor types for detecting GI-tract diseases. A relatively recent experimental 13-sensor multi-technology e-nose instrument, the Warwick olfaction system or WOLF e-nose (composed of a sensor array with eight amperometric electro-chemical (EC) sensors, two nondispersive infrared optical devices, and a single photo-ionization detector), was developed with the capability to discriminate between CRC and IBS with

broad overlapping symptoms by analysis of lower and higher molecular weight urine headspace volatiles with linear discriminant analysis [1].

A nondispersive infrared sensor optical e-nose, the Warwick Optical Electronic Nose, was very recently developed for healthcare applications at point-of-care facilities [29]. This instrument contains four tunable, optical infrared sensors, IR range (3.1 to 10.5 μm), based on the detection principle of differential molecular absorption of IR-radiation by sample VOCs that absorb at specific IR-frequencies. IR adsorption by VOCs results in a decrease in IR-signal, resulting in specific sensor responses to different VOC gas mixtures present in sample headspace. Hitherto, this experimental e-nose has been tested with six small molecular weight VOCs, but not for detection of GI-tract diseases.

5. Conclusions

The development of novel portable electronic-nose devices offers new potential opportunities and tools to simplify and speed up point-of-care clinical diagnostic processes and help facilitate more rapid noninvasive early detection of GI-diseases which should allow earlier more effective treatments that improve patient prognoses and significantly shorten recovery times following treatments.

Acknowledgments: The author has received allocated research funds, available for technology transfer of new disease detection and diagnostic technologies, from the USDA Forest Service, Southern Research Station, to cover the costs of publishing in open access journals.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Westenbrink, E.; Arasaradnam, R.P.; O'Connell, N.O.; Bailey, C.; Nwokolo, C.; Bardhan, K.D.; Covington, J.A. Development and application of a new electronic nose instrument for the detection of colorectal cancer. *Biosen. Bioelectronics* **2015**, *67*, 733–738, doi:10.1016/j.bios.2014.10.044.
2. Arasaradnam, R.P.; Covington, J.A.; Harmston, C.; Nwokolo, C.U. Review article: next generation diagnostic modalities in gastroenterology – gas phase volatile compound biomarker detection. *Aliment. Pharmacol. Ther.* **2014**, *39*, 780–789.
3. de Meij, T.G.; Larbi, I.B.; van der Schee, M.P.; Lentferink, Y.E.; Paff, T.; sive Droste, J.S.T; 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.
4. de Groot, E.F.; de Meij, T.G.; Berkhout, D.J.; van der Schee, M.P.; de Boer, N.K. Flatography: Detection of gastrointestinal diseases by faecal gas analysis. *World J. Gastrointest. Pharmacol. Ther.* **2015**, *6*, 111–113.
5. Sagar, N.M.; Cree, I.A.; Covington, J.A.; Arasaradnam, R.P. The interplay of the gut microbiome, bile acids and volatile organic compounds. *Gastroent. Res. Practice* **2015**, *2015*, 1–6.
6. Wilson, A.D. Recent progress in the design and clinical development of electronic-nose technologies. *Nanobiosens. Dis. Diagn.* **2016**, *5*, 15–27, doi:10.2147/ndd.s66278
7. Wilson, A.D. Electronic-nose devices – Potential for noninvasive early disease-detection applications. *Ann. Clin. Case Rep.* **2017**, *2*, 1401.
8. Ahmed, I.; Greenwood, R.; Costello, B.de L.; Ratcliffe, N.M.; Probert, C.S. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. *Plos One* **2013**, *8*, e58204.
9. Wilson A.D. Biomarker metabolite signatures pave the way for electronic-nose applications in early clinical disease diagnoses. *Curr. Metabolom.* **2017**, *5*, 90–101, doi:10.2174/2213235X04666160728161251
10. 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.
11. Wilson, A.D.; Baietto, M. Applications and advances in electronic-nose technologies. *Sensors* **2009**, *9*, 5099–5148, doi:10.3390/s90705099
12. Wilson, A.D.; Baietto, M. Advances in electronic-nose technologies developed for biomedical applications. *Sensors* **2011**, *11*, 1105–1176, doi:10.3390/s110101105
13. Wilson, A.D. Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath. *Metabolites* **2015**, *5*, 140–163, doi:10.3390/metabo5010140
14. de Groot, E.F.J., de Meij, T.G.J.; van der Schee, M.P.; de Boer, N.K.H. Letter: volatile metabolomics of exhaled breath or faecal gas? *Aliment. Pharmacol. Ther.* **2015**, *41*, 698–707.

15. Probert, C.S.J.; Jones, P.R.H.; Ratcliffe, N.M. A novel method for rapidly diagnosing the causes of diarrhea. *Gut* **2004**, *53*, 58–61.
16. Patel, N.; Alkhoury, N.; Eng, K.; Cikach, F.; Mahajan, L.; Yan, C.; Grove, D.; Rome, E.S.; Lopez, R.; Dweik, R.A. Metabolomic analysis of breath volatile organic compounds reveals unique breathprints in children with inflammatory bowel disease: A pilot study. *Aliment. Pharmacol. Ther.* **2014**, *40*, 498–507.
17. Arasaradnam, R.P.; Covington, J.; Nwokolo, C.U. Editorial: metabolomics analysis of breath volatile organic compounds – a new scent for inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **2014**, *40*, 732–733.
18. Ahmed I., Greenwood R., Costello B., Ratcliffe N., Probert C.S. Investigation of faecal volatile organic metabolites as novel diagnostic biomarkers in inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **2016**, *43*, 596–611, doi:10.1111/apt.13522.
19. Arasaradnam, R.P.; McFarlane, M.; Daulton, E.; Skinner, J.; O'Connell, N.; Wurie, S.; Chambers, S.; Nwokolo, C.U.; Bardhan, K.; Savage, R.; Covington, J.A. Non-invasive exhaled volatile organic biomarker analysis to detect inflammatory bowel disease (IBD). *Digest. Liver Dis.* **2016**, *48*, 148–153.
20. Arasaradnam, R.P.; McFarlane, M.J.; Ryan-Fisher, C.; Westenbrink, E.; Hodges, P.; Thomas, M.G.; Chambers, S.; O'Connell, N.; Bailey, C.; Harmston, C.; Nwokolo, C.U.; Bardhan, K.D.; Covington, J.A. Detection of colorectal cancer (CRC) by urinary volatile organic compound analysis. *Plos One* **2014**, *9*, e108750.
21. Arasaradnam, R.P.; Westenbrink, E.; McFarlane, M.J.; Harbord, R.; Chambers, S.; O'Connell, N.; Bailey, C.; Nwokolo, C.U.; Bardhan, K.D.; Savage, R.; Covington, J.A. Differentiating coeliac disease from irritable bowel syndrome by urinary volatile organic compound analysis - a pilot study. *Plos One* **2014**, *9*, e107312.
22. 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.
23. Peng, G.; Hakim, M.; Broza, Y.Y.; Billan, S.; Abdah-Bortnyak, R.; Kuten, A.; Tisch, U.; Haick, H. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Brit. J. Cancer* **2010**, *103*, 542–551.
24. de Meij, T.G.J.; de Boer, N.K.H.; Benninga, M.A.; Lentferink, Y.E.; de Groot, E.F.J.; van de Velde, M.E.; van Bodegraven, A.A.; van der Schee, M.P. Faecal gas analysis by electronic nose as a novel, non-invasive method for assessment of active and quiescent paediatric inflammatory bowel disease: proof of principle study. *J. Crohn's Colitis* **2014**, *8*, 91–106, doi:10.1016/j.crohns.2014.09.004.
25. Shepherd, S.F.; McGuire, N.D.; de Lacy Costello, B.P.; Ewen, R.J.; Jayasena, D.H.; Vaughan, K.; Ahmed, I.; Probert, C.S.; Ratcliffe, N.M. The use of a gas chromatograph coupled to a metal oxide sensor for rapid assessment of stool samples from irritable bowel syndrome and inflammatory bowel disease patients. *J. Breath Res.* **2014**, *8*, 026001, doi:10.1088/1752-7155/8/2/026001.
26. McGuire, N.D.; Ewen, R.J.; Costello, C.D.; Garner, C.E.; Probert, C.S.J.; Vaughan, K.; Ratcliffe, N.M. Towards point of care testing for *C. difficile* infection by volatile profiling, using the combination of a short multi-capillary gas chromatography column with metal oxide detection. *Meas. Sci. Technol.* **2014**, *25*, 065108.
27. Berkhout; D.J.C.; Niemark, H.J.; Buijck, M.; van Weissenbruch, M.M.; Brinkman, P.; Benninga, M.A.; van Kaam, A.H.; Kramer, B.W.; Andriessen, P.; de Boer, N.K.H.; Meij, T.G.J. Detection of sepsis in preterm infants by fecal volatile organic compounds analysis: a proof of principle study. *J. Ped. Gastroent. Nutri.*, **2017**, *65*, e47–e52.
28. de Meij, TG.; van der Schee, M.P.; Berkhout, D.J.; van de Velde, M.E.; Jansen, A.E.; Kramer, B.W.; van Weissenbruch, M.M.; van Kaam, A.H.; Andriessen, P.; van Goudoever, J.B.; Niemark, H.J.; de Boer, N.K. Early detection of necrotizing enterocolitis by fecal volatile organic compounds analysis. *J. Pediatr.* **2015**, *167*, 562–567.
29. Esfahani, S.; Covington, J.A. Low cost optical electronic nose for biomedical applications. In Proceedings of the Eurosensors 2017 Conference, Paris, France, 3–6 September 2017; pp. 589.

