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Clinical applications of breath testing

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Abstract

Breath testing has the potential to benefit the medical field as a cost-effective, non-invasive diagnostic tool for diseases of the lung and beyond. With growing evidence of clinical worth, standardization of methods, and new sensor and detection technologies the stage is set for breath testing to gain considerable attention and wider application in upcoming years.

Introduction and context

With each breath exhaled thousands of molecules are expelled, providing a window into the physiological state of the body. The utilization of breath as a medical test has been reported for centuries as demonstrated by Hippocrates in his description of fetor oris and fetor hepaticus in his treatise on breath aroma and disease [1]. Even in modern times clinicians have noted distinct changes in the breath odor of patients with specific diseases such as diabetes, renal failure, and hepatic diseases [2-4]. However, it was Linus Pauling's milestone discovery of 250 unique substances present in exhaled breath that offered promising insight into breath testing [5]. Since this discovery, breath analysis has rapidly evolved as a new frontier in medical testing for disease states in the lung and beyond [1]. Breath analysis is now used clinically to monitor asthma, diagnose transplant organ rejection, diagnose *Helicobacter pylori* infection, detect blood alcohol concentration, and monitor breath gases during anesthesia, mechanical ventilation, and respiration, among numerous other applications [1,6,7].

Recent advances

Breath analysis may offer a relatively inexpensive, rapid, and non-invasive method for detecting a variety of diseases. With recent advancements in mass spectrometry (MS) and gas chromatography MS (GC-MS), it is possible to identify thousands of unique substances, such as volatile organic compounds (VOCs) and elemental gases, in the breath [8]. Improved technologies such as selected-ion flow-tube MS (SIFT-MS), multi-capillary column ion mobility MS (MCC-IMS), and proton transfer reaction MS (PTR-MS) have provided real time, precise identification of trace gases in human breath in the parts per trillion range [9-11]. On the other hand, unlike traditional quantitative breath analysis, the electronic nose is essentially trained to recognize odor patterns using an array of gas sensors. The electronic nose has shown accuracy in the detection of lung cancer, pneumonia, and asthma with specificities and sensitivities ranging from 74-98%, as well as in the discrimination between diseases such as chronic obstructive pulmonary disease and asthma [12-15]. Table 1 provides a selected list of the growing number of technologies being applied to breath testing.

More recent technological advancements in breath analysis have moved beyond measuring volatiles in the gas phase into measurement of semivolatiles and compounds dissolved in aerosolized droplets in exhaled breath condensate (EBC) and in exhaled breath vapor (EBV). Aerosolized droplets in EBC can be captured by a variety of methods and analyzed for a wide range of biomarkers, such as metabolic end products, proteins, cytokines, and chemokines, with expanding possibilities [16,17]. With 3000 volatile compounds identifiable using EBC and twice the volatile metabolite concentration compared to traditional breath gas analysis, this application has the potential to provide superior information about

Table 1. Current breath-based test technologies

Spectrometry	Gas chromatography	Other
Mid-infrared absorption spectroscopy	Automated thermal desorption gas chromatography mass spectrometry (ATD-GC-MS)	Chemiluminescense
Multi pass cell-laser absorption spectroscopy	Gas chromatography flame ionization detection (GC-FID)	Electrochemical cell sensor technology
Tunable diode laser absorption spectroscopy (TDLAS)	Gas chromatrography mass spectrometry (GC-MS)	Gas sensor array technology
Cavity ring-down spectroscopy (CRDS)	Photoionization detection gas chromatography	Nanosensor technology
Cavity leak-out spectroscopy (CALOS)	Solid phase microextraction gas chromatography mass spectrometry (SPME-GC-MS)	Infrared and para-magnetic sensors
Cavity enhanced optical frequency comb spectroscopy	Gas chromatography time of flight mass spectrometry (GC-TOF-MS)	Calorimetry
Integrated cavity output spectroscopy (ICOS)	Gas chromatography differential mobility spectrometry (GC-DMS)	Sol-gel sensor technology
Laser magnetic resonance spectroscopy (LMRS)	Gas chromatography ion mobility mass spectrometry (GC-IMS-MS)	Laser based sensor technology
Laser photoacoustic spectroscopy	Gas chromatography/Fourier transform ion cyclotron resonance mass spectrometry (GC/FT-ICR-MS)	
Faraday-LMRS	Gas-liquid partition chromatography (GLPC)	
Selected ion flow tube mass spectrometry (SIFT-MS)	Gas chromatography electrolyzer-powered flame ionization detector (GC-EFID)	
Proton transfer reaction mass spectrometry (PTR-MS)		
Faraday modulation spectroscopy		
Ion trap (2D) and (3D) mass spectrometry		
Time of flight mass spectrometry (TOF-MS)		
Isotope ratio mass spectrometry (IR-MS)		
Multi-capillary column ion mobility mass spectrometry (MCC-IMS)		
High sensitivity (hs)-PTR-MS		
Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS)		
Proton transfer reaction time of flight mass spectrometry (PTR-TOF-MS)		

breathprints of healthy and disease states [8,18]. EBV sampling has also yielded promising results as a new breath sampling method. EBV sampling pre-concentrates breath samples using a solid-phase microextraction fiber inserted into a modified RTube™, a common device also used in EBC sampling. This procedure provides the potential advantages of faster breath sampling and analysis, increased portability, minimal user training, use in contaminated environments, and no requirement for a power source. EBV sampling may yield additional compounds not detected in EBC and may provide greater sensitivity as a sampling method, expanding the spectrum of breath sampling [19].

Implications for clinical practice

The science of breath analysis is rapidly expanding, the technology is improving, and several new applications have been developed or are under commercial development. A major breakthrough over the past decade has been the increase in breath-based tests approved by the US Food and Drug Administration (FDA). Devices measuring common breath gases: oxygen, nitrogen, water vapor, and carbon dioxide in patient respiratory monitoring have served as a platform for technological growth in clinical breath testing applications. In particular, earlier devices, such as those providing the detection of blood alcohol concentration, *H. pylori* infection, lactose intolerance, and airway monitoring by end-tidal carbon dioxide, have demonstrated clinical benefits as well as diagnostic success in clinical breath testing. Table 2 provides a selected list of the breath-based tests currently approved by the FDA.

One recent landmark in clinical breath testing occurred in 2003 when the FDA approved the first device that measures the fraction of exhaled nitric oxide (FE_{NO}) for asthma monitoring. The desktop NIOX® (currently NIOX® FLEX) was followed by a handheld NIOX® MINO device (both by Aerocrine, Inc., Solna, Sweden) that received FDA clearance in 2008. Advantages provided by FE_{NO} monitoring devices include its non-invasive nature, ease of repeat measurements, and use in adult and child populations with severe airflow obstruction where other techniques would be difficult or impossible to perform [20]. FDA approval of these devices has largely been attributed to the standardization

Table 2. Breath-based tests approved by the US Food and Drug Administration [33]

Molecule detected	Disease/condition	Trade name of analysis instrument	Technology	Manufacturer	FDA approval date
CO_2, O_2, N_2O	Respiration	Consolidated-Nier model 21-201 isotope ratio mass spectrometer	Dual inlet system gas isotope ratio mass spectrometer	Consolidated Electrodynamics Corporation, Inc., Pasadena, CA, USA	Before 28 May 1976
CO_2	Respiration	Tidal Wave® Carbon Dioxide Monitor, Model 610	Sensor technology	Novametrix Medical Systems, Inc., Wallingford, CT, USA	20 November 1996
H_2	Lactose malabsorption	Micro H2	Sensor technology	MICRO DIRECT, Inc., Auburn, ME USA	24 January 1997
^{13}C, ^{18}O, CO_2, ^{15}N, N_2, NO_2	Respiration	ABCA-NT System	Continuous flow gas isotope ratio mass spectrometer	Europa Scientific, Ltd. Concord, MA USA	16 December 1997
CO_2, O_2, N_2O and anesthetic agents	Respiration, anesthesia	Datex-Ohmeda Compact Airway Module M-CAiOVX and M-COVX	Infrared sensor, paramagnetic sensor	Datex-Ohmeda, Inc., Tewksbury, MA, USA	23 August 2000
$^{13}CO_2/^{12}CO_2$	*H. pylori*	UBiT®-IR3000 Infrared Spectrometry System	Infrared (IR) spectrophotometer	Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan	21 December 2001
O_2, CO_2	Respiration	BSM-4100A	Sensor technology	Nihon Kohden America Inc., Foothill Ranch, CA, USA	24 October 2000
$^{13}CO_2/^{12}CO_2$	*H. pylori*	BreathTek™ - UBiT® UBT for *Helicobacter pylori*	UBiT® IR spectrophotometer	Meretek Diagnostics, Inc., Nashville, TN, USA	17 January 2002
O_2, CO_2, N_2O, anesthetic agents	Respiration, anesthesia	Ag-920PA	Sensor technology	Nihon Kohden America Inc., Foothill Ranch, CA, USA	25 July 2002
O_2, CO_2, N_2O, anesthetic agents and gases	Respiration, anesthesia	BSM-5130A Series Bedside Monitor	Sensor technology	Nihon Kohden America, Inc., Foothill Ranch, CA, USA	March 04, 2003
NO	Asthma, airway inflammation	NIOX®	Chemiluminescence	Aerocrine AB, Solna, Sweden	30 April 2003
CO_2	Respiration, anesthesia	Datex-Ohmeda S/5 Single-Width Airway Module M-miniC	MiniCO2 IR measuring sensor	Datex-Ohmeda, Needham, MA, USA	23 April 2003
(C4-C20) alkanes, monomethylalkanes	Grade 3 heart transplant rejection	Heartsbreath	Gas chromatography mass spectrometry	Menssana Research, Inc., Fort Lee, NJ, USA	24 February 2004
H_2	Lactose malabsorption	Micro H2 Breath Monitoring Device with HYDRA Software Utility	Electrochemical gas sensor	Micro Medical Ltd., Kent, UK	19 May 2004
$^{13}CO_2/^{12}CO_2$	*H. pylori*	POCone Infrared Spectrophotometer	IR spectrophotometer	Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan	15 July 2004
Alcohol	Breath alcohol	AlcoMate CA2000 Digital Alcohol Detector	Semiconductor oxide sensor	KHN Solutions LLC, San Francisco, CA, USA	11 August 2004
Alcohol	Breath alcohol	AlcoHAWK Precision™ Digital Alcohol Detector	Semiconductor oxide sensor	Q3 Innovations, LLC, Eagan, MN, USA	9 February 2005
CO_2	Ventilation	C-CO2™	Colorimetric carbon dioxide sensor	Marquest Medical Products, Inc., Englewood, CO, USA	1 March 2005
CO_2	Ventilation	Datex-Ohmeda S/5™ Single-width airway module, E-miniC	Narrow band IR sensor	GE Healthcare, Needham, MA, USA	14 October 2005
Alcohol	Breath alcohol	AL-6000 Breath Alcohol Tester	Semiconductor oxide sensor	Sentech Korea Corp., Kyeonggi-do, Korea	11 May 2006
Alcohol	Breath alcohol	AL-5000 Breath Alcohol Tester	Semi-conductive alcohol sensor	Sentech Korea Corp., Kyeonggi-do, Korea	30 October 2006
Alcohol	Breath alcohol	Breath Alcohol .02 Detection System	Electrochemical analyzer	Akers Biosciences, Inc., Thorofare, NJ, USA	18 December 2006

(Continued)

Table 2. Breath-based tests approved by the US Food and Drug Administration [33] (*Continued*)

Molecule detected	Disease/condition	Trade name of analysis instrument	Technology	Manufacturer	FDA approval date
CO_2	Respiration	OLG-2800A	Sensor technology	Nihon Kohden America, Inc., Foothill Ranch, CA, USA	27 December 2006
Alcohol	Breath alcohol	BACTRACK® Breath Analyzer	Semiconductor oxide sensor	KHN Solutions LLC, San Francisco, CA, USA	14 September 2007
CO_2	Respiration, anesthesia	EMMA™ Emergency Capnometer	IR gas analysis	Phasein AB, Danderyd, Sweden	28 December 2007
CO	CO poisoning, carboxy-haemoglobin	EC50 ToxCO+	Electrochemical gas sensor technology	Bedfont Scientific Ltd., Rochester, Kent, UK	21 February 2008
NO	Asthma, airway inflammation	NIOX MINO®	Electrochemical sensor	Aerocrine AB, Solna, Sweden	3 March 2008
NO	Asthma, airway inflammation	Apieron Insight eNO	Sol-gel-heme protein sensor	Apieron, Inc., Menlo Park, CA, USA	14 March 2008
Alcohol	Breath alcohol	AlcoHAWK® PT500 Digital Alcohol Detector	Fuel cell sensor	Innovations, LLC, Independence, IA, USA	25 July 2008
CO_2	Ventilation	Nihon Kohden TG-970P Series CO2 Sensor Kit	IR absorption spectrometry	Nihon Kohden America, Inc., Foothill Ranch, CA, USA	2 March 2009
Alcohol	Breath alcohol	BACTRACK® Select Breathalyzer Model (S30, S50, S70)	Semiconductor (Si) oxide sensor	KHN Solutions LLC, San Francisco, CA, USA	19 March 2009
Alcohol	Breath alcohol	BACTRACK® Select Breathalyzer Model S80	Fuel cell electrochemical sensor	KHN Solutions LLC, San Francisco, CA, USA	24 March 2009

of clinical FE_{NO} monitoring and detection via breath analysis [21]. In order for this simple yet powerful tool to achieve its potential, we need to further understand the roles that FE_{NO} and similar biomarkers of disease play in different clinical settings and across populations, and their specific functions in disease.

A recent clinical application of breath testing has been in the diagnosis of lung cancer. Currently, clinicians rely on relatively expensive and invasive diagnostic tests, such as computed tomography exams, chest radiography, sputum analysis, and lung biopsies, which remain largely ineffective in early stage lung cancer diagnosis. Researchers have demonstrated success using trained dogs in the breath diagnosis of both early and late stage lung cancers with sensitivities and specificities approaching 99%, providing promise for future lung cancer breath tests [22]. Breath testing may provide a promising alternative diagnostic tool for lung cancer as evidenced by numerous studies with specificities and sensitivities ranging from 71-94% [14,23-28]. However, in order to be useful as an upfront screening test for high-risk populations, as a tool to evaluate pulmonary nodules, or as a diagnostic test for lung cancer, a breath test should be at least 90-95% sensitive and specific [29].

As the field of breath research has developed over the past decade, the need for standardization in sampling has grown. Attempts at sampling only critical portions of exhaled breath have proven successful by using end-tidal sampling, as evidenced by finding VOC concentrations most reflective of compounds dissolved in the blood [30]. End-tidal sampling (collecting breath only at the end of exhalation) has shown success over mixed expiratory sampling (collecting the entire exhaled breath) because samples are less likely to be diluted by mixing with dead space volume (inspired air not taking place in gas exchange) and ambient air. A useful application is buffered end-tidal on-line sampling, which measures VOC breath concentrations over a large mass range quickly and uses multiple MS technologies, such as SIFT-MS and PTR-MS, for breath analysis [31]. It is also promising because it uses on-line sampling (the sampling device is connected to the analytical device) versus less accurate off-line sampling (the sample is collected and later brought to the analytical device using reservoirs such as Tedlar® bags). Device calibration and validation have helped by accounting for exogenous VOCs and ambient air contamination in the sampling environment [19]. Since detection of many VOCs occurs at the parts per billion and parts per trillion levels, it is essential to control for exogenous sources of VOCs because ingestion of certain foods, medications, gut bacterial flora, and exposure to

chemicals and pollution, amongst many other things, will alter VOCs in exhaled breath [32]. It is important for researchers to consider the change in the concentration of several VOCs in disease states as well as the utility of ranking systems for VOC predictability and new methods for accounting for ambient VOC sources, such as calculating alveolar gradients [8,28]. Despite receiving considerable attention in recent years, issues with standardization have been a major limitation of clinical breath testing. This has been evidenced by difficulties in establishing baseline VOC concentrations and the wide range of results represented in the literature for VOC concentrations in disease. Thus, it is necessary in the future to search for innovative methods for breath research.

There are numerous potential advantages for breath analysis as a clinical test. The method is non-invasive (the sample is relatively easy and painless to acquire), the sample is likely to be rich with information (a single test can scan for signatures of many abnormalities or markers of disease), it has the potential for low cost, and lends itself to easy administration. The field of breath testing has grown tremendously in recent years and with evolving technologies in sampling, sensor design, standardization, and analytical methods breath analysis has the potential to clinically benefit individuals on a global scale in the future.

Abbreviations

EBC, exhaled breath condensate; EBV, exhaled breath vapor; FDA, US Food and Drug Administration; FE_{NO}, fraction of exhaled nitric oxide; GC-MS, gas chromatography MS; MCC-IMS, multi-capillary column ion mobility MS; MS, mass spectrometry; PTR-MS, proton transfer reaction MS; SIFT-MS, selected-ion flow-tube MS; VOC, volatile organic compound.

Competing interests

The authors declare that they have no competing interests.

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