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Cellular Respiration, Metabolomics and the Search for Illicit Drug Biomarkers in Breath: Report from PittcCon 2017

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Introduction:

The contemporary arena of breath research has become much broader than merely exploring volatile organic compound (VOC) biomarkers in exhaled air. Although breath-borne VOCs still dominate – and intrinsically shape – the field, there is a wide variety of topics that are now included in “breath related” research, and these have been diversely featured at the Pittsburgh Conferences on Analytical Chemistry and Applied Spectroscopy (Pittcon) over the past decade. This year, at the Pittcon Conference and Expo 2017, the major focus areas were based on the broad concept of cellular respiration (cell breath), the use of breath-based metabolomics for clinical applications, and the search for viable breath tests to establish driving impairment due to drug use, primarily cannabis. In addition, several other topics were covered, including analytical improvements in breath analysis and breath physiology. The exposition portion of Pittcon included a number of new instrumental developments that may ultimately provide improved analytical methods for trace level analysis of breath volatiles and aerosols. Members of the International Association of Breath Research (IABR; www.breathinternational.com) organized and participated in two invited symposia and two conferee networking sessions, plus a series of analytical presentations in related topic areas.

Background:

The annual Pittcon meeting is a convenient venue for gathering together a wide range of researchers and analytical equipment manufacturers that may both provide and gain benefit from the more focused topics of breath research. Members of IABR have regularly participated in Pittcon meetings by presenting talks and organizing different types of sessions. In addition, Journal of Breath Research has been publishing annual meeting reports (such as this one) reviewing new technologies and studies related to breath measurements since 2010.

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Most recently, we have discussed National Security and Intelligence Applications at Pittcon 2016 in Atlanta, GA, Adverse Outcome Pathways for Toxicity Testing at Pittcon 2015 in New Orleans, LA, and Human Microbiome and Exposome Diagnostics at Pittcon 2014 in Chicago, IL [1–3].

These reports serve an important purpose in continually documenting the most recent thoughts, advances and consensus in breath research, and we continue this tradition here with a report on the most recent Pittcon, held from 4–9 March 2017 in Chicago, IL. As such, these reports are important tools in bringing contemporary thought to the forefront of breath research.

Pittcon 2017 comprised many hundreds of technical presentations, symposia, short courses, and conferee networking sessions, as well as an extensive exposition of analytical instrumentation and equipment. In this report, we limit the discussions to breath-related topics, analytical methodologies and recent advancements that we expect to be of particular interest to the *J Breath Res* readership.

Technical symposia and sessions:

This year, there were four major technical sessions fully devoted to breath research related topics, plus a scattering of other bioanalytical and instrumentation oriented presentations that impact breath research. Here we will discuss those that stood out to the authors, but do not promise that this discussion is fully exhaustive.

The main breath sessions were two symposia, one on cellular respiration and another on breath-based metabolomics, and two conferee networking sessions, one on non-invasive biomedical analysis and the other on detection of drug abuse in human breath. These are discussed individually in this section.

Symposium: Cellular Respiration

Joachim Pleil (US EPA, NC) and Jane Hill (Dartmouth College, NH) created and organized a symposium entitled “*Cellular Respiration (Breath-Based) Metabolomics: In Vitro Links to Living Systems for Toxicology, Food Safety, Infection, Pharmaceutical Production and Metabolism Diagnostics*”. This session was intended to cover a wide variety of topics that had cellular level organic analysis as a common theme. Human exhaled breath is comprised of a complex mixture of VOCs as well as vapors and aerosols that originate at the cellular level or from external exposures [4]. In recent years, the question has arisen about the particular source for different constituents, which is important for assessing exposures, diagnosing disease states, and for understanding human metabolism. One approach for understanding the total breath profile is to employ a reductionist strategy wherein we start with individual cell lines *in vitro* to determine subsets of compounds that are directly linked to those cells [5]. In fact, this strategy has become an extension of traditional breath research, with the contemporary state of knowledge on cellular respiration being published in a collection of invited articles in a special issue of *J Breath Res* entitled “*Focus Issue on the Cellular Origin of Volatile Metabolites*”, guest-edited by Wojciech Filipiak and Jane Hill [6].

An initial lecture in the cellular respiration symposium set the scene of applying analytical *in vitro* approaches to human metabolome discovery. Subsequent lectures focused on specific cases, such as how these methods could be applied to certain disease states, for example, cystic fibrosis [7], or for toxicity assessments [5]. Moreover, research on cellular respiration is not merely restricted to human host origin; other talks in this session included how characteristic VOC emissions might be utilizable as markers for food spoilage [8] or pharmaceutical (fermentation) production yield [9], as screening or process analytical technology tools.

Symposium: Breath Metabolomics

The second of the invited symposia “*Metabolomics: Breath as a Sample for Clinical Analysis*”, was initiated and chaired by Michael Costanzo (Breathtec Biomedical, Vancouver, BC) and Richard Yost (University of Florida, FL). Compared to the aforementioned symposium, this session had a strong clinical focus and was geared more towards what one might consider being ‘traditional’ breath analysis. The session commenced with a comprehensive overview of the current state of breath research, the challenges faced, but also the manifold opportunities it presents [10]. This was complemented by another lecture on the pitfalls in breath biomarker analysis and how the community should strive towards quantitative analyses of identified compounds and an understanding of their biochemical production pathways, rather than focus too heavily on data mining of complex breath matrices containing innumerable unknown compounds [11]. An overview lecture on the environmental impact of exogenous compounds on exhaled breath highlighted a further complexity in breath research, namely the ubiquitous presence of confounders, but conversely offered ideas of how this might be exploited to learn of perturbations that might lead to adverse outcomes in health [12]. A lecture outlining the successful uses of breath constituents to flag diverse lung and systemic diseases offered the promise of how breath analysis might ultimately find its way into the clinical setting [13]. This was complemented by a lecture on potential point-of-care diagnostic screening approaches using newly-developed, miniaturized, on-line analytical equipment via field asymmetric ion mobility spectrometry (FAIMS) [14].

Conferee Networking: Non-Invasive Biomedical Analysis

The Conferee Networking sessions serve to gather together researchers interested in particular topics and to provide a forum for guided and informal discussions. This year, Wolfram Miekisch (Rostock University Medical Center, Germany) arranged and hosted one such session entitled “*Non-invasive biomedical analysis: VOCs in the air – from cellular metabolism to crowd monitoring*”.

After a short introduction on potential sources of VOCs (cellular, microbial, systemic, environmental) the discussion centered on the question of the conditions under which breath biomarkers are suitable for use in biomedical analysis. It is now generally accepted within the breath research community that individual unique biomarkers for individual diseases are unlikely to exist, as suggested by independent clinical studies [15]. However, it is certainly possible that pattern shifts of common metabolites (biomarkers) could become aggregate indicators of preclinical disease states. On a discussion around this topic, the participants

agreed that concentration changes of relevant VOCs might gain further importance and be more effective for clinical screening applications. The ensuing discussion covered diverse breath-related topics, from the effects of non-disease related vectors on exhaled VOC concentrations, e.g., physiologically-induced changes [16–19], the microbiome [1], and previous/acute environmental exposure [2]. In addition, different methodologies and instrumentations were discussed, as was the pressing need for standardization and comparability of different methods for breath sampling and VOC analysis. The roundtable discussion concluded that further indepth studies at all tiers (*in vitro*, *in vivo* and crowd-based) – as well as their crosslinks – are desirable to understand the complex behavior of VOC biomarkers and to foster biomedical applications. Of particular relevance in this context are the analytical methods that allow VOCs to be monitored in real-time, such as proton-transfer-reaction mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS) [20]. In this way, a broad range of applications such as the monitoring of bacterial or cellular growth *in vitro* and *in vivo* [21] or physiological processes [22], diseases progression and therapy control [23], as well as the assessment of pharmaceutical or illicit drugs [24] and human environmental exposures [25] might be realized.

Conferee Networking: Detection of Drug Consumption in Human Breath

The other breath-based Conferee Networking session dealt with a growing application of interest for breath, namely the detection of illicit narcotics consumption in exhaled breath. The session, entitled “*Detection of Drug Consumption in Human Breath*”, which was organized by Maggie Tam (Canada Border Services Agency) and Wolfgang Vautz (Ion-Gas GmbH, Dortmund, Germany), set out with a discussion that focused on cannabis use. This drug is of particular interest due to its widespread decriminalization or even legalization of possession in a growing number of states in the USA as well as various countries throughout the world. Moreover, the trend towards ‘medical marijuana’ prescriptions presents a concerning development in terms of driving under compromised cognitive function. The alcohol breathalyzer is perhaps the most well-known breath test to the general public – and indeed for most people confronted with the topic of breath research, it is the specific application of identifying drink-drivers that will immediately spring to mind. Law enforcement agencies in states and countries that have decriminalized or legalized cannabis face a huge challenge in catching drug-divers, and current tests require extensive and costly analyses, not withstanding their inability to clearly identifying recent versus previous drug abuse, due to the latency of target compounds in the body. The discussion in this session focused heavily on what is needed to help law enforcement to screen for drug use – not just illicit but potentially also prescription drugs – and covered topics from analytical tools to legislation required. Issues raised included the potential differences between smoking and ingesting cannabis, as well as cross sensitivities and confounders. It was unanimously agreed that any on-the-spot breath-based test would need to deliver immediate results.

Related breath-based topics:

Oral presentations on breath research in otherwise non-breath-focused individual sessions were abundant. These included a look at applications for (qualitative) comprehensive

analysis of human breath for the detection of the most common drugs by gas chromatography ion-mobility-spectrometry (GC-IMS), or marijuana detection using capillary microextraction of volatiles, a comparison of VOC patterns between nasal and oral breathing, as measured in real-time using PTR-time-of-flight-MS (PTR-TOF-MS) [19], and a comparison of different microextraction techniques [26] for pre-concentrating VOCs from bacterial culture headspace. Further, the potential factors affecting *in vitro* VOC measurements over biological cultures, as well as a novel analytical set-up to overcome related challenges.

Exhibition:

A major feature of Pittcon is the extensive exhibition of vendors, manufacturers, and providers of services for the analytical chemistry community. Of particular note in the context of this report are the various equipment manufacturers who provide new methodologies and hardware to push the frontiers of possibilities for breath related analyses of all types. In addition, the attendees have the opportunity to interact with the scientists from the various companies to engage in detailed discussions regarding specific technical issues, as well as learn what is in the works for the future. An extremely useful feature of the exhibition is the element of surprise; sometimes one can walk around the corner of a booth and find a novel piece of lab-ware, or an interesting new twist on an instrument that can engender a new approach for the laboratory back at home.

Summary:

A Pittcon conference is always an interesting experience for all researchers, regardless of their particular scientific niche. For us, the practitioners of breath research, Pittcon provides a mixture of tangentially related topics that can help clarify certain technical approaches, or even suggest new directions. At the 2017 meeting, the two new breath-related fields, cellular respiration and breath metabolomics, attracted a great deal of attention from scientists that would perhaps not attend a purely breath focused meeting such as the annual IABR summit. This is important for the continual growth of the breath research field, and also to monitor the pulse of broader scientific and technical trends.

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