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Meeting Report:

Breath Biomarkers Networking Sessions at PittCon 2010, Orlando Florida

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Background

The Pittsburgh Conference and Exposition, or "PittCon" (http://www.pittcon.org/), is one of the largest international conferences for analytical chemistry and instrumentation typically attracting about 25,000 attendees and 1,000 commercial exhibitors. PittCon began in 1950 as a small spectroscopy and analytical chemistry gathering and, as the name implies, originated in Pittsburgh, Pennsylvania. The conference has outgrown its humble beginnings and is now held in early March every year in the United States' largest convention centers rotating among Chicago Illinois, Atlanta Georgia, Orlando Florida and New Orleans Louisiana. In addition to the instrumentation exposition, PittCon offers over 2,000 technical presentations, thousands of poster presentations, numerous plenary lectures, and specialty workshops for analytical instrumentation. Recently, the organizers have implemented a series of "Networking Sessions" as an extra opportunity for attendees to share expertise and opinions to cope with the technological explosion of innovations of new subspecialties.

Breath Sessions at PittCon

For the 2010 meeting, two networking sessions (on different days) featured breath analytical technologies and applications. The first, entitled ***"Breath Tests in Medicine"***, was organized and facilitated by Dr. Michael Phillips, M.D. (mphillips@menssanaresearch.com), from Menssana Research in Newark, NJ who also serves as a Professor of Clinical Medicine at New York Medical College. Dr. Phillips is well-known for his research in breath tests for diagnostic medicine and for the HeartsBreath Test approved by the U.S. Food and Drug Agency for early diagnosis of heart transplant rejection. The second session, entitled ***"No Needles No Pins: New Directions for Non-invasive Drug Monitoring and Biomarker Determination"*** was hosted by Dr. Wolfram Miekisch, Ph.D., (wolfram.miekisch@uni-rostock.de) from the Rostock University Hospital in Rostock Germany. Dr. Miekisch develops analytical methods for measuring biochemicals in exhaled breath from patients to develop non-invasive diagnostic tests for critical care medicine.

Dr. Phillips' session: This session focused on the development of breath methods for the diagnosis of disease and the acceptance of such methods in the medical community. It was arranged as an introduction by Dr. Phillips followed by four short (< 5 min) presentations from invited speakers interspersed with about 20 min of participant discussions.

The overall concept of measuring volatile organic biomarkers in breath was introduced by Dr. Phillips who reprised the history of medical breath analysis starting with Hippocrates and Lavoisier. He then presented his concepts for bringing new medical tests into mainstream acceptance by outlining the progression from laboratory method to clinical instrument to home care monitor. He also suggested the use of a dome structure for capturing patients' exhaled breath from their direct breathing zone rather than a traditional mask or mouthpiece to relieve concerns about infection. The participants' discussions revolved around the practicality vs. sample quality issues of using "stand-off" breath collection. The primary concerns were the mixing of ambient air using the dome concept. The primary advantage was deemed to be the broader ability to capture breath from babies and from otherwise breathing impaired patients. Regardless of technique used, however, the group agreed that for breath analysis to penetrate further into mainstream diagnostic medicine that the community needs to provide specific guidance and acceptance criteria for sample validation.

The concerns regarding the environmental influences on the interpretation of exhaled breath data were presented by Dr. Joachim Pleil (pleil.joachim@epa.gov) from the U.S. Environmental Protection Agency. He cautioned that external influences could mask or mimic a true biological response from the patient and that environmental exposures could result not only from inhalation in the clinic, but also from dermal contact, non-dietary ingestion, and food consumption, all of which would be unknown to the physician. He presented examples of the uptake and elimination of environmental chemicals and how they could vary in time depending upon hypothetical previous exposures. He further discussed that the immediate environment of the patient is influenced by metabolic compounds from other people so that there is never a true "zero" concentration for inspired air. The participants agreed that this was a crucial concern and discussed their own experiences. The common thread was the inconsistency of cross-laboratory results for the case-control experiments for developing breath biomarkers for specific diseases. The consensus was that the breath community needs to develop guidance not only for the collection and analyses processes, but also for interpreting environmental interferences, possibly with multiple sampling times during the clinical observation period of patients.

A myriad of technical advances and needs were presented by Prof. Ed Overton (ebovert@lsu.edu) from Louisiana State University. He explained his concept for all breath analysis instrumentation as a progression of three distinct tasks: sample focus, separation, and detection. He then described the tiers of instrumentation starting with highly sensitive and specific in-laboratory instruments employing gas-chromatography – mass spectrometry (GC-MS) through the hope for an inexpensive clinic or at-home monitor possibly based on chemical sensor technology. In the subsequent discussion, the participants were concerned that lowering costs for home care instrumentation may not be achievable using existing mass detection based approaches and there were suggestions made regarding alternate detection schemes including optical and immunochemistry approaches. Dr. Overton laid out the challenge to the community of developing a one-dimensional GC type instrument for under $20,000 for disease diagnosis.

The focus was subsequently shifted to the practicality of bringing breath science to specific measurement needs. Mr. David Legendre (david.legendre@lackland.af.mil) from the U.S. Air Force presented his recent efforts of matching U.S. Department of Defense (DoD) resources to in-field diagnostic solutions. In particular, he expressed current concerns for the rapid breath detection of influenza, tuberculosis, and recent contact with explosives. The participants asked about various time elements required to address DoD issues; there was ultimately a long

discussion regarding anecdotal disease diagnosis by medical personnel based on breath odor and how such experience could be implemented in developing new point of care instrumentation. Mr. Legendre solicited the participants for new research and development ideas that could be funded by the Air Force.

The last discussion revolved around the difficult task of breath biomarker discovery; Dr. Mark Libardoni (mark_libardoni@leco.com) from LECO Corporation presented recent progress using 2-dimensional gas chromatography (GC x GC) followed by high speed time-of-flight mass spectrometry (ToF-MS). He showed computer generated 3-dimensional representations of breath analyses and indicated that they have detected over 1,300 discrete organic compounds in a single sample. The current strategy for discovery is to overlay case and control samples and use an "omics" approach for teasing out differences; compounds that are differentially expressed are then deemed candidates as biomarkers of the disease. This led to a long discussion among the participants regarding specific identification and pattern recognition; that is, does one need to unambiguously identify biomarker compounds or can one rely on a mass spectrum based library match, or even on retention time and base peak comparison. Although no true consensus was reached, the group agreed that from a clinical perspective, it may be sufficient to use empirical differences to distinguish pre-clinical disease state patients from controls, however, many felt that identifying specific biomarkers is necessary to understand the biochemical pathways leading to disease and to subsequently develop medical intervention and prevention strategy.

To wrap up the session, Dr. Phillips led a brief discussion revisiting the concerns over direct vs. stand-off breath sampling and solicited the participants for their thoughts on the subject. The group briefly discussed the potential for breath analysis beyond cancer diagnosis, and suggested breath measurement as an outpatient diagnostic technique for general practitioners for screening for common colds, strep throat, sinus infection, etc. to assure proper prescription of medication. Dr. Phillips commented that from the perspective of point of care clinical practice, empirical methods for pattern recognition are sufficient to make a valid diagnosis and suggested that knowledge of the specific biochemical pathways may not be necessary. As time ran out, Dr. Phillips thanked everyone for their contributions to the session and encouraged them to stay in touch.

Dr. Miekisch's session: This session centered on the hospital and clinical application of breath testing "as close as possible" to the patient. It was introduced by Dr. Miekisch who proceeded to elicit response from the group using three brief presentations by invited speakers interspersed with discussions.

The focus of this session, as explained by Dr. Miekisch, was on the implementation of non-invasive (and minimally invasive) methods for drug monitoring and disease detection. He described the limitations (e.g. complexity, space) and the potential advantages (e.g. rapid intervention) of inserting diagnostic activity directly into the clinical setting. He explained that any analytical instrumentation brought into the operating room or intensive care unit must be as automated, unobtrusive, and simple as possible to use; physicians and nurses are focused on the life of the patient, not on the operation of unfamiliar equipment.

The next presentation was given by Prof. Jochen Schubert, M.D. (jochen.schubert@med.uni-rostock.de) from the University of Rostock (Germany) Hospital where he serves as the director of critical care medicine. He gave a brief history of medicine that began with empirical (trial and error) treatments with plant derived extracts and subsequently progressed over time adding

antibiotics, anaesthesia, surgery, imaging, biochemical analysis, and molecular genetics. He then focused on the issues of clinical chemistry and described the current needs of the modern critical care physician, that is, the rapid turn-around of medical measurements of drug levels to efficiently adjust dose, and the continuous monitoring for patient health state using biomarkers that may be indicative of infection or allograft rejection. The participants then discussed various aspects of exhaled breath analysis that could be implemented as well as the limitations when compounds of interest may not be sufficiently volatile to be detected in breath.

In response to this last point, Dr. Miekisch gave a brief presentation regarding new technologies for monitoring infused anaesthetics such as propofol or antibiotics such as linezolid and daptomycine. He explained that the standard procedures require 5 to 10 ml blood draws followed by laboratory analyses; he then described a much faster method for extracting the compound of interest directly from the blood through adsorption onto a fiber inserted into the vein. These fibers can be easily swapped out through a venous catheter ("hep-lock") and rapidly assayed via methanol extraction and liquid-chromatography (LC) analysis. Although somewhat more invasive than breath analysis, this methodology does not require cumulative blood loss from repeat sampling (which is important especially for small babies) and could be implemented via an existing hep-lock that is already in-place in critically ill patients. The group discussed this new technology and agreed that this is a very interesting approach for minimizing the effect on the patient and serves as an excellent compromise for compounds that could not be detected in breath. The participants also discussed the possibility of non-invasive monitoring of non-volatile chemicals using saliva measurements as in current use for illicit drugs. The consensus was that this is worth further consideration for clinical medicine, but that current methods for saliva analysis are more geared towards a yes/no answer and may not be quantitative enough for adjusting therapeutic dose.

The session discussions then returned to breath analysis and another technical innovation was introduced by Mr. Dietmar Hein (dietmar.hein@pas-tec.com) from PAS Technologies, Magdala, Germany. He described adsorbent filled syringe needles that can be used as a breath collection device and then subsequently as the delivery system for analysis. The method was initially developed by Drs. Schubert and Miekisch at the University of Rostock and has been commercialized by PAS with an auto-injector compatible with various commercially available gas chromatography – mass spectrometry (GC-MS) systems. Mr. Hein presented results from a series of breath studies and concluded that samples as small as 10 - 50 ml pre-concentrated on the needle device were sufficient to achieve excellent reproducibility for common breath constituents. The participants asked about calibration strategies for this new technology and then considered various headspace methods and linear response methods using incrementally sampled volumes.

The discussion then shifted to available analytical methods; the group considered various existing technologies and agreed that breath analysis via GC x GC ToF is considered the gold standard. They conceded, however, that this is requires complex and expensive instrumentation and is much better suited for biomarker discovery than for high-throughput analysis. Furthermore, regardless of the analytical sensitivity and accuracy, the group cautioned that breath analysis is only as good as the sample itself; breath sample concentrations are subject to pulmonary gradients, inhalation/exhalation cycles, and differences in frequency and volume among patients. Consistency in collection was deemed of utmost importance.

This led to further exchange regarding the dichotomy regarding the use of empirical patterns for detecting disease as opposed to fully understanding the biochemical pathway of each biomarker used for diagnostic assessments. An example was brought out regarding a recent case-control study that identified isopropanol as a breath biomarker for disease that may very likely have been an environmental artifact from disinfection products. The medical contingent agreed that knowing the biochemistry is a laudable goal, but that often the practicality of quickly identifying patients at risk outweighs such academic ambitions.

Dr. Andreas Hengstenberg (andreas.hengstenberg@draeger.com) from Draeger, Luebeck, Germany, described new non-invasive point-of-care instrumentation based on electrochemical sensors. He showed data comparing breath analyses with standard blood analyses for propofol, a common human anaesthetic. The results showed equivalent analytical quality, but the electrochemical instrument had the advantage of providing real-time results. The subsequent discussion revolved around issues of volatility of various anaesthetics and the applications of breath analysis. The main anaesthetics used in surgery (isoflurane, desflurane, and sevoflurane) are volatile chemicals and delivered via the inhalation pathway; as such they are amenable to breath monitoring. Propofol is a newer compound that has advantages during recovery (less side-effects) and is preferred for certain patient phenotypes, however, it is delivered through intravenous infusion and has very low partition into breath. Existing monitoring methods for propofol require blood draws and therefore the new non-invasive breath application is a very exciting development for the medical community.

At the conclusion of the session, Dr. Miekisch summed up the various comments and presentations. He reiterated his viewpoint that unequivocal identification of proposed biomarkers and their biochemical origin is required for a valid breath test. He then proceeded to describe his thoughts regarding the tiers of technology required to achieve a truly "diagnostic breath test" that range from: controlled breath sampling and appropriate laboratory analysis; interpretation of data including pharmacokinetics, biology, and confounders; development of fast on-site analysis; and at the pinnacle, achieving a completely automated clinical instrument. He suggested (tongue in cheek) that the ultimate goal of breath diagnostic medicine would be an instrument akin to the "Tricorder" made famous by Dr. "Bones" McCoy and Mr. Spock of the Star Trek television series. He then thanked the group for their participation and encouraged everyone to continue to interact.

Concluding remarks

The two disparate sessions had some remarkable commonalities. Discussion in both concluded that breath samples are subject to environmental exposure artifacts that can compromise diagnostic interpretation and that future research needs to consistently address this issue. Participants in both sessions also agreed that GC x GC ToF is the current gold standard for breath biomarker discovery but that there is value in more modest GC-MS analytical techniques for routine analysis, and that ultimately, inexpensive clinical outpatient and "at-home" monitors for breath analysis need to become a mainstream medical diagnostic tool. Another recurrent theme was pattern based vs. biochemical based disease diagnosis, however, this topic defied consensus in both sessions. Finally, participants in both sessions recognized that consistent sampling of breath is a non-trivial challenge due to mixing with tracheal dead-volume and because exhalation is dependent on the individual patient's breathing rate and depth of

inhalation. Regardless of the technology used, normalizing the sampling procedure was considered crucial in comparing results across institutions.

The emergence of well-attended sessions regarding breath analysis at a large, eclectic conference such as PittCon 2010 is a great step forward for the discipline. It has particular value as a launching point for technical exchange as the primary purpose of PittCon is to present new technologies and analytical instrumentation to the scientific community; articulating the technical issues for breath analysis in an instrumentation intensive environment will foster future collaboration as well as inserting breath analysis into the collective consciousness of the equipment manufacturers as a viable application.

The United States Environmental Protection Agency through its Office of Research and Development has subjected this article to Agency administrative review and approved it for publication.

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