5th & 6th
November

Breath Biopsy Conference 2024

Providing an opportunity for the global breath research community to come together and discuss the latest advances and challenges in breath research

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Breath Biopsy Conference 2024

Day 1 (5th November)

13:00 GMT Welcome

13:00	**Plenary Talk**	**Dr Oleg Schelochov - Genome Research Institute** Breath Biopsy in Inborn Errors of Metabolism: A Proof-of-Principle Study in Propionic Acidemia
13:30	**Microbiome and Breath Based VOCs**	**Dr Matteo Tardelli - Owlstone Medical** Analyzing Postprandial Response Using VOCs in Exhaled Breath: A Longitudinal Study Design **Dr Andrew Kau - Washington University School of Medicine** The breath volatilome is shaped by the gut microbiota **Dr Joshua Bates & Dr Riccardo Avvisati - Owlstone Medical** Developing Portable Breath Analyzers for Deployment into the Clinic or At Home
15:00	**Applications of Breath Analysis**	**Dr Audrey John - Children's Hospital of Philidelphia, Research Institute** Talk Title TBC **Daniela Polag - Heidelberg University** What does breath methane tell us?
16:00	**Sponsored Talk LECO**	**Dr Flavio Antonio Franchina - University of Ferrara** Evaluation of sampling conditions for volatile metabolites in exhaled breath combining trap enrichment, dual-stage thermal desorption, comprehensive two-dimensional gas chromatography, and time-of-flight mass spectrometry (TD-GC×GC-TOFMS)
16:15		**Poster Session**
16:30	**Standardization in Breath Analysis and Building a VOC Atlas**	**Dr. Arghavan Nawaby - US Food & Drug Administration** An Aim to Standardize Breath Analysis – Developing Regulatory Science Tools **Dr. Eric Miller - US Food & Drug Administration** Confident Identification of Breath Matrix Compounds: An RST to Serve the in vitro Diagnostic Industry **Dr Omar Rivera - US Food & Drug Administration** Evaluating Method Performance for Breath Analysis

Day 2 (6th November)

11:00 GMT Welcome

11:00 — **Early Careers**

Mark Woollam - Integrated Nanosystems Development Institute
Establishing Healthy Baselines to Harness the Potential of Breath-based Biomarkers for Non-invasive Health Monitoring in Diverse Medical Applications

Yuta Matsuoka - Kyoto University
Volatile oxidized lipids generated via metal-dependent lipid peroxidation of ω-6 PUFAs are breath biomarkers for monitoring ferroptosis in vivo

Michal Skawinski - Maastricht University
The in vitro effects of different bread types on fecal microbiome composition and volatile metabolic activity in healthy and Non-Coeliac Wheat Sensitive subjects

12:00 — **Poster Session**

13:00 — **Sponsored Talk Thermo Fisher Scientific**

Dr Kamila Schmidt - Manchester University
Offline breath analysis: storing up trouble?

13:15 Lunch

14:15 — **Markes International, SepSolve Analytical**

Dr Helen Martin - Markes International
Advancing breathomics workflows: Innovations in thermal desorption to support large scale studies

14:30 — **Standardization in Breath Analysis and Building a VOC Atlas**

Julia Greenwood - Owlstone Medical
Volatile Biomarker Discovery and the VOC Atlas

Dr. Kavita M. Jeerage - National Institute of Standards and Technology
Potential Uses of Breath Surrogates for the Development and Deployment of New Infectious Disease Breathalyzers

15:45 — **Panel Discussion**

Standardization in Breath Analysis and building a VOC Atlas

Hosted by Billy Boyle, CEO and Co-Founder Owlstone Medical

Joined by:
James Covington - Professor in Electronic Engineering, University of Warwick
Brooke Kaiser - Principal Investigator, Pacific Northwest National Laboratory
Iain White - Assistant Professor, University of Manchester
Ben de Lacy Costello- Associate Professor Biosensing and Diagnostics, University of Bristol

Sponsors:

Markes International & SepSolve Analytical

Established in 1997, Markes International is a global manufacturer and supplier of specialist analytical instrumentation, accessories, supplies and consumables that enhances the sensitivity and application scope of GC–MS. Their products help analysts worldwide detect trace-level volatile and semi-volatile organic compounds (VOCs and SVOCs) in solids, liquids and gases.

Watch their talk on 5th November

Advancing breathomics workflows: Innovations in thermal desorption to support large scale studies

Dr Helen Martin
Business Unit Manager,
Thermal Desorption

MARKES international

SepSolve Analytical

Sponsored Talk:

Speaker Biography

As Thermal Desorption Business Unit Manager at Markes International, Helen oversees research and development, application development and product marketing for the TD instrument range. Helen joined Markes International in 2012 as a technical support specialist and has spent time working in new product development and product marketing. Before starting her career with Markes, Helen gained a PhD from Loughborough University in clinical metabolomics, during which her primary research focus was developing non-invasive sampling strategies for breath, skin, and saliva for analysis by thermal desorption with GC-MS.

Talk Absract

Helen Martin1, Laura Miles1, Aaron Davies1 and Jessica Berger1
1. Markes International Ltd, 1000B Central Park, Western Avenue, Bridgend, CF31 3RT, UK

Breath-based biomarker discovery is advancing rapidly, with many studies scaling up to large clinical trials and identifying viable candidate marker compounds. As sample sizes increase, so do the study durations and personnel requirements, underscoring the need for reliable automation, high throughput methods, and rigorous quality control. Thermal desorption (TD) coupled with gas chromatography-mass spectrometry (GC-MS) is the gold standard for breath analysis, particularly suited for large-scale trials due to its compatibility with remote sampling, ease of sample storage and shipment, and high automation.
In this presentation, we introduce new instruments designed to streamline workflows and reduce the quality control burden in high throughput laboratories and longitudinal studies. The Re-collect 10™ enables splitting a single sample onto up to 10 sorbent tubes, allowing for the simultaneous generation of 10 replicate quality control or calibration standards, saving over an hour of analysts' time per set. Additionally, it facilitates the creation of pooled biological samples with representative matrices for quality control, inter-laboratory studies, and method development. The Dual-TDTM system doubles the productivity of TD-GC systems by enabling simultaneous processing and analysis of two samples, ideal for high throughput labs needing to verify the cleanliness of sorbent tubes or sampling apparatus.

These advancements not only streamline workflows but also ensures consistency and reliability across different study conditions and locations, ultimately pushing the boundaries of biomarker research and clinical application.

Sponsors:

Thermo Fisher Scientific

Thermo Fisher Scientific instruments, equipment, software, services and consumables empower scientists to solve for complex analytical challenges in pharmaceutical, biotechnology, academic, government, environmental and industrial research, as well as the clinical laboratory. Their products address a range of needs from sample, material characterization, and chemical analysis to clinical diagnoses and biological-based therapeutics manufacturing.

Watch their talk on 6th November

"Offline Breath Analysis: Storing Up Trouble?"

Dr Kamila Schmidt

Manchester University

ThermoFisher SCIENTIFIC

Sponsored Talk:

Speaker Biography

Kamila earned her PhD at the University of Salford in 2016, where she researched volatile metabolites as potential biomarkers for lung cancer via in vitro investigation of cells, under the supervision of Prof. Ian Podmore. Kamila then joined the Manchester Institute of Biotechnology as a Research Technician in LC-MS analytics. There, she worked on projects related to the LC-MS-based untargeted metabolomics for chassis engineering of antibiotics-producing bacteria and the molecular signalling governing soil microbiome interactions, in the group led by Prof. Eriko Takano. She now works as a lab manager in the Centre for Precision Approaches to AMR (PI: Stephen Fowler, Tim Felton) and leads the clinical translation of biomarkers using mass spectrometry, including breath volatiles.

Talk Abstract

Sample storage is an essential consideration for study design in clinical research. It is especially important for studies with multiple sampling sites and repeated visits over months or years. Offline breathomics studies lack consensus on sorbent tube storage time and temperature, and as such sampling and analytical confounders are introduced when analysing data. In this study, we explore the effect of storage time and temperature on breath samples collected in parallel using the ReCIVA device (Owlstone Medical, Cambridge, UK). Samples were analysed using TD-GC-Orbitrap MS. The ability to store samples for long periods of time will increase confidence in biomarker discovery and reduce the effect of confounders such as analytical drift and batch effects.

Sponsors:

LECO

Since 1936, LECO Corporation has pioneered new approaches in analytical instrumentation and is renowned for the quality of its instruments and support. Committed to innovation, LECO made a significant move into the mass spectrometry field with the introduction of the first commercial GC-TOFMS, the Pegasus, in 1995. Later, they integrated ultra-fast TOF technology with comprehensive two-dimensional GC (GCxGC). LECO is dedicated to delivering the highest-performing GCxGC-TOFMS systems available, and in April 2024, they released their third-generation benchtop series instrument, the BTX.
LECO's commitment to quality, continuous innovation, and customer support makes them the ideal partner for those at the forefront of scientific discovery and testing. Making it easy to use the latest advances in GCxGC TOFMS to drive research and analysis in clinical, forensic, toxicology, and environmental laboratories.

Watch their talk on 5th November

'Evaluation of sampling conditions for volatile metabolites in exhaled breath combining trap enrichment, dual-stage thermal desorption, comprehensive two-dimensional gas chromatography, and time-of-flight mass spectrometry (TD-GC×GC-TOFMS)'

Dr Flavio Antonio Franchina

University of Ferrara

LECO
EMPOWERING RESULTS

Sponsored Talk:

Speaker Biography

Flavio Antonio Franchina is Associate Professor in Analytical Chemistry at Department of Chemical, Pharmaceutical, and Agricultural Sciences of the University of Ferrara, since 2021. His research is devoted to the development and validation of advanced analytical techniques (chromatographic and mass spectrometric) for the evaluation of complex matrices of various origins and within the context of clinical research (breath analysis, pathogens in vitro volatile metabolites, data analysis etc.). Besides the numerous participations at international meetings and papers published in high-impact journal in the field of analytical chemistry, his international experience and scientific merit have been recognized with the John Phillips award for the outstanding contributions to the field of multidimensional GC, and the presence in the power list 'Top 40 Under 40' in analytical science by The Analytical Scientist, in which he was listed twice, in 2018 and 2022.

Talk Absract

Riccardo Di Stefano1, Marco De Poli1, Luisa Pasti2, Alberto Cavazzini1, Flavio A. Franchina1
1. University of Ferrara, Dept. of Chemistry, Pharmaceutical and Agricultural Sciences, Ferrara, Italy
2. University of Ferrara, Dept. of Environmental and Prevention Sciences, Ferrara, Italy

Exhaled breath analysis is suffering from a lack of standardized sampling and analysis procedures, impacting the robustness of inter-laboratory results, and thus hampering proper external validation. The aim of this work was to verify compliance and validate the performance of a comprehensive two-dimensional gas chromatography - mass spectrometry system (GC×GC-MS) by monitoring probe metabolites in exhaled breath. These probe analytes were selected after the ingestion of coffee or a digestive pill.
The GC×GC-MS system herein used consisted of the following features: I) a quad-jet thermal modulator to ensure the high-sensitivity and high-separation power needed for the analysis of complex biological samples and trace metabolites; II) a time-of-flight MS combining high sensitivity, proper data-point density for the fast modulated peaks and spectral consistency which enables reliable deconvolution; III) an auxiliary pressure joint located downstream of the injector to allow an adequate dry purge of the tubes without affecting columns and detector performance; IV) an online thermal desorption unit, constituted by a fast heating/cooling inlet body and a cold trap, allowing an efficient dual-stage thermal desorption of metabolites from the trap tubes into the GC×GC-MS system.
We measured the VOCs sampling performance of different adsorbent materials for thermal desorption, in the context of clinically relevant samples. Specifically, we used 7 different adsorbents, packed singularly and in combination, on Fetal Bovine Serum (FBS) and human breath spiked with probe analytes. For the comparison, we selected a mix of 19 standards to evaluate the sensitivity and repeatability between the different adsorbents.

Sponsors:

Syft Technologies

Syft was founded in 2002 and has over 150 professionals in 7 countries. Syft is considered the world leader in real-time, direct injection mass spectrometry with more than 20 years of SIFT-MS expertise. Syft instruments support a broad range of industries worldwide including pharma and CDMOs, environmental protection, consumer goods, food, flavor and fragrance, semiconductor manufacturing and many more. Continually developed and proven in high stakes commercial environments, you can be assured of operational robustness, speed and support.

Plenary Speaker

Plenary Talk:

Dr Oleg Shchelochkov

Breath Biopsy in Inborn Errors of Metabolism: A Proof-of-Principle Study in Propionic Acidemia

Dr Shchelochkov is a board-certified pediatrician, clinical geneticist and medical biochemical geneticist, and is currently an associate investigator at NHGRI. After his early pediatrics training at the University of Iowa and genetics training at Baylor College of Medicine, Dr Shchelochkov was a tenure-track assistant professor within the Pediatrics Department and Division of Genetics at the University of Iowa Hospitals and Clinics. He has been part of the NHGRI research community since 2015, starting as a staff clinician. Until recently, he was an associate research-physician studying many aspects of organic acidemias. In 2021, NHGRI appointed Dr Shchelochkov as the new Director of Clinical and Laboratory Residencies and Fellowships. Dr Shchelochkov is an author and a co-author on more than 60 peer-reviewed publications and book chapters.

Abstract

Breath Biopsy in Inborn Errors of Metabolism: A Proof-of-Principle Study in Propionic Acidemia

Background: Impaired oxidation of branched chain amino acids may give rise to volatile organic compounds (VOCs). We hypothesized that VOCs will be present in exhaled breath of participants with propionic acidemia (PA), and their relative abundance would correlate with characteristics of the disease.

Methods: We enrolled 5 affected participants from a natural history study of PA (ClinicalTrials.gov ID NCT02890342) plus five age- and sex-matched unaffected controls. We collected exhaled breath using a non-invasive breath sampling platform paired with thermal desorption-gas chromatography-mass spectrometry. Clinical and biochemical parameters were correlated with the relative abundance of VOCs.

Results: Unbiased screening identified several candidate VOC biomarkers of PA. One candidate putatively identified as 3-pentanone was the most abundant (45-fold higher in cases vs. controls, p-value < 0.05). 3-Pentanone abundance positively correlated with plasma propionylcarnitine ($p = 0.01$), plasma 2-methylcitrate ($p < 0.05$), 3-OH-propionate ($p < 0.01$), full scale IQ ($p < 0.01$), and showed a statistical trend with height z-scores ($p = 0.08$). It inversely correlated with the whole-body in vivo oxidation of 1-13C-propionate ($p < 0.05$). In a participant who received an orthotopic liver transplant, 3-pentanone levels were lower and segregated with “mild” PA.

Conclusion: Non-invasive breath sampling is a promising method to identify and quantitate VOCs that correlate with the clinical and biochemical parameters of PA. Our proof-of-principle findings may have wide implications for the diagnosis and severity stratification of inborn errors of metabolism affecting oxidation of amino acids which might be monitored in a similar fashion.

Microbiome Extended Session

Dr Andrew Kau

Division of Allergy and Immunology and Center for Women's Infectious Disease Research at Washington University School of Medicine

The breath volatilome is shaped by the gut microbiota

I am a physician-scientist in the Division of Allergy and Immunology and Center for Women's Infectious Disease Research at Washington University School of Medicine. My basic and translational research program is directed at understanding how consortia of human microbes that colonize our mucosal surfaces shape human health, particularly in their ability to modify immunity. We are currently engaged in three major, ongoing projects:

- Defining mechanisms by which the gut microbiota influence lung health. We have shown that human gut microbes alter susceptibility to asthma through their ability to alter gut barrier function. We have further expanded on these findings to investigate the gut microbial origins and function of volatile organic compounds found in human breath.

- Investigating the functions of airway commensals in allergic disease. Our prior work has shown that the composition of the airway microbiota in individuals with asthma is enriched for certain species, including Haemophilus. We are presently testing how microbial metabolites produced by Haemophilus influence airway inflammation.

- Developing mucosal vaccines to target uropathogens within the gut. Previous work supports the notion that bacteria that cause urinary tract infections (UTIs) originate in the gut. Based on this observation, we are developing new vaccine strategies to reduce uropathogen colonization in the gut and, ultimately, protect against UTIs.

Abstract:

The breath volatilome is shaped by the gut microbiota

The gut microbiota is widely implicated in host health and disease, inspiring translational efforts to implement our growing body of knowledge in clinical settings. However, the need to characterize gut microbiota by its genomic content limits the feasibility of rapid, point-of-care diagnostics. The microbiota produces a diverse array of xenobiotic metabolites that disseminate into tissues, including volatile organic compounds (VOCs) that may be excreted in breath. We hypothesize that breath contains gut microbe-derived VOCs that inform the composition and metabolic state of the microbiota. To explore this idea, we compared the breath volatilome and fecal gut microbiomes of 27 healthy children and found that breath VOC composition is correlated with gut microbiomes. To experimentally interrogate this finding, we devised a method for capturing exhaled breath from gnotobiotic mice. Breath volatiles are then profiled by gas-chromatography mass-spectrometry (GC-MS). Using this novel methodology, we found that the murine breath profile is markedly shaped by the composition of the gut microbiota. We also find that VOCs produced by gut microbes in pure culture can be identified in vivo in the breath of mice monocolonized with the same bacteria. Altogether, our studies identify microbe-derived VOCs excreted in breath and support a mechanism by which gut bacterial metabolism directly contributes to the mammalian breath VOC profiles.

Dr Matteo Tardelli

Owlstone Medical

Analyzing Postprandial Response Using VOCs in Exhaled Breath: A Longitudinal Study Design

Matteo is a Senior Biomarker Scientist in Owlstone Medical's Clinical and Translational Science program. With 12+ years' experience in rare diseases, metabolic disorders, and immunology, his expertise spans academic research and bio/med-tech and management consulting. He is an accomplished project leader, excelling in team-oriented environments for pre- and clinical biomarker discovery.

Abstract:

Analyzing Postprandial Response Using VOCs in Exhaled Breath: A Longitudinal Study Design

Volatile organic compounds (VOCs) in exhaled breath can reflect metabolic responses, and understanding the intricate connection between human metabolism and different physiological states in a non-invasive manner is crucial for future clinical applications. In this single-center pilot study, we recruited 20 volunteers who underwent an overnight fast followed by a standardized meal challenge. Two breath samples were collected during the fasting state and two at 20 minutes and 1-hour post-meal, and analyzed using thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) with the Breath Biopsy OMNI Platform. As anticipated, several exposure compounds related to food and flavoring, such as valencene, limonene, and terpenes like alpha-pinene and beta-pinene, significantly increased 20 minutes after food consumption and stabilized by the 1-hour mark. Notably, several microbiome-related short-chain fatty acids (SCFAs), including butyric acid, propionic acid, and acetic acid, exhibited an upward trend following meal intake. Other microbiome-related metabolites, such as 1-propanol and 2,3-butanedione, also increased, while indole and 3-methylindole—products of oral microbial fermentation of amino acids like tryptophan—decreased or remained unchanged post-meal. Eleven VOCs associated with fasting glucose levels, including 3-methylindole, corroborated findings from a previous metabolomic study (with seven inversely and four positively correlated). Non-invasive identification of diet-induced metabolic changes in breath VOCs will enhance future research related to diseases.

Dr Joshua Bates and Dr Riccardo Avvisati

Owlstone Medical

Developing Portable Breath Analyzers for Deployment into the Clinic or At Home

Joshua is a Senior Systems Engineer in Owlstone Medical's Engineering and Operations department. His undergraduate degree was in Medical Engineering, and joined us after completing his PhD. He now works on developing our portable hydrogen and methane breath analyzer for research and diagnostic purposes.

Riccardo is a Senior Data Scientist at Owlstone Medical, and works on analyzing and processing data for our gastrointestinal projects and OMED Health. This includes data from the OMED Health portable breath analyzer.

Abstract:

Developing Portable Breath Analyzers for Deployment into the Clinic or At Home

There is a preference for the development of portable, point-of-care (PoC) devices capable of analyzing the breath of patients rapidly, without the need to transport samples to the laboratory. However, the development of such tests relies upon a solid understanding of the relevant compounds in breath, the mechanisms contributing to their presence, and the connection between their concentration and specific diseases. We have recently developed the OMED Health Breath Analyzer which utilizes metal oxide sensors to accurately measure hydrogen and methane in the breath as key biomarkers of gut microbiome activity with comparable accuracy to currently used in-clinic hydrogen and methane testing equipment. This device could support the diagnosis, longitudinal monitoring of treatment response and compliance, and recurrence of gastrointestinal conditions such as small intestinal bacterial overgrowth (SIBO), and intestinal methanogen overgrowth (IMO). This device was developed using a synthetic breath system, built to match specifications taken from Atlas-type data. The VOC Atlas could therefore provide a reference database to outline specifications for sensors and instrumentation companies can use to develop portable VOC breath analyzers.

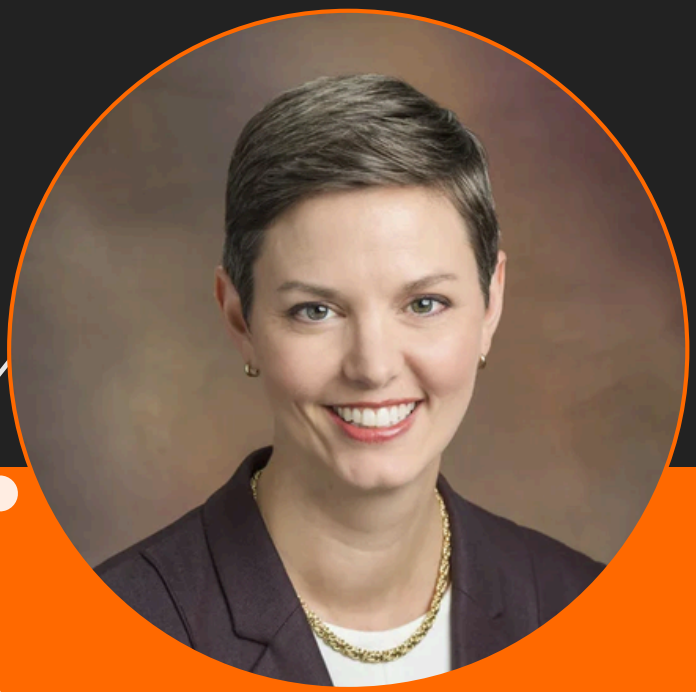
Applications of Breath Analysis

Dr Audrey John

Children's Hospital of Philidelpia, Research Institute

Title To Be Confirmed

A physician-scientist, Dr John's NIH-funded research focuses on the Plasmodium falciparum malaria parasite, with a particular interest in understanding its basic molecular and cellular biology and functions of its specific metabolic pathways — what the parasite needs to make and why it needs to make it — to identify new antimalarial drug targets and develop new diagnostics. Dr John is an investigator in the Pathogenesis of Infectious Diseases of the Burroughs Welcome Fund. She has received numerous accolades, including awards from the American Chemical Society, March of Dimes, and, most recently the IDea Incubator Grand Prize from the Infectious Diseases Society of America.



Abstract:

Talk Absract

Dr Daniela Polag

Heidelberg University

What does breath methane tell us?

Dr Daniela Polag has been a Research Associate in the Biogeochemistry research group at the Institute of Earth Sciences, Heidelberg University, since 2014. Her academic journey began with a Diploma in Geophysics from Johann-Wolfgang-Goethe-University in Frankfurt, completed between 1996 and 2001. She then served as a Scientific Associate at the Institute of Applied Geophysics, Technical University Berlin, from 2003 to 2006. Dr Polag then pursued her PhD as part of the 'Radiometry' research group under the Heidelberger Academy of Sciences at the Institute of Environmental Physics, Heidelberg University, from 2006 to 2009. From 2010 to 2013, she was a Research Associate with the 'ORCAS' research group at the Max-Planck-Institut for Chemistry in Mainz.

Dr Polag's research is centered on the energetic use of CO2 to enhance methane yield during biogas production from sewage sludge and renewable energy crops. Her expertise includes stable carbon isotope analysis during anaerobic digestion, isotope labeling experiments, online monitoring of δ13C in methane and CO2 using laser absorption spectrography, and breath CH4 research.

Abstract:

What does breath methane tell us?

Methane (CH4), the simplest carbon-based volatile organic compound, is a common constituent of exhaled breath, typically ranging from 20 parts per billion by volume to 40 parts per million (ppmv) above atmospheric background values (around 2 ppmv). Breath CH4 has long been associated exclusively with anaerobic microbial activity (methanogenesis) in the gastrointestinal tract and is thought to be primarily related to intestinal motility. In recent years, our comprehensive high-resolution and high-precision studies of breath CH4, including stable carbon and hydrogen isotope measurements (δ13C-CH4 and δ2H-CH4 values) have broadened the picture of breath CH4 formation. Analytical techniques include gas chromatography equipped with a flame-ionization detector or, isotope ratio mass spectrometer, or optical methods such as cavity ring down spectroscopy.

In a cross-sectional study, we found that the dominant factor of high breath CH4 (> 4 ppmv) is age, or rather age-related changes in human physiology which needs to be considered as a confounding factor in potential CH4 diagnostics. A long-term longitudinal study of individuals who experienced immunological events during the measurement period (i.e., infections, inflammation, induced perturbation by vaccination) showed that CH4 dynamics (temporal increase or decrease in breath CH4 production) are closely related to immune reactions. Isotopic patterns of δ13C-CH4 and δ2H-CH4 values and the application of stable isotope tracing techniques served as a proof of concept for CH4 degradation and the cellular CH4 formation via a radical-driven process, respectively. Our observations imply that non-invasive breath CH4 measurements have the potential to be applied as a biomarker for oxidative-reductive stress reactions.

However, additional investigations are required to obtain unambiguous evidence of non-microbial CH4 formation in humans and the underlying processes of its generation. This will be a significant challenge because in the case of high emitters—where CH4 formation by methanogens is the dominant process—it is difficult to distinguish between the non-microbial and microbial pathways of CH4 production. Although the biochemical pathways of CH4 formation and degradation are not yet fully understood, frequent monitoring of breath CH4 levels may be used to detect significant changes in ROS levels and oxidative stress, and could potentially be used as an additional diagnostic tool in the field of system biology and precision medicine.

Early Careers

Mark Woollam

Integrated Nanosystems Development Institute

Establishing Healthy Baselines to Harness the Potential of Breath-based Biomarkers for Non-invasive Health Monitoring in Diverse Medical Applications

Mark Woollam, Senior Research Scientist at Integrated Nanosystems Development Institute (INDI), is a researcher at Indiana University Indianapolis (IUI) focusing on developing methods to sample and identify volatile organic compound (VOC) biomarkers in exhaled breath. He received his Bachelor of Science degree in Chemistry from Indiana University of Pennsylvania in 2017 and joined IUI in the same year for his doctoral studies in the field of bioanalytical chemistry. Woollam completed his PhD in the summer of 2022, where he concentrated on utilizing chemometric approaches to identify VOC biomarkers (in urine and breath) for prostate cancer, breast cancer, hypoglycemia, COVID-19 and cystic fibrosis. Upon graduation, he joined INDI where he continued to drive scientific discovery, as demonstrated through publishing articles in high-impact factor journals and helping secure several extramurally funded awards.

Abstract:

Establishing Healthy Baselines to Harness the Potential of Breath-based Biomarkers for Noninvasive Health Monitoring in Diverse Medical Applications

Noninvasive monitoring of breath-based biomarkers, namely volatile organic compounds (VOCs), has immense potential to improve public health through increased screening and diagnosis of medical conditions. Many studies have identified novel biomarkers in exhaled breath, but a challenge that still exists is that healthy baseline levels of VOCs have not been benchmarked. To help address this, cross-sectional and longitudinal breath samples were collected and analyzed through solid phase microextraction coupled to gas chromatography-mass spectrometry. To ensure volunteers were “relatively healthy”, subjects also donated blood samples to perform comprehensive metabolic panels and completed a food diary/lifestyle questionnaire. In this study, 164 different individuals provided samples cross-sectionally, and ten of these volunteers donated a total of ten longitudinal samples each over the course of six months. The results showed there was a core set of 32 VOCs that were comprised of diverse functional groups including terpene/-oids, carbonyls, and aromatics. Hierarchical heatmaps were produced for the cross-sectional data, which revealed that there may be a relationship between VOC structure and expression in breath. Monoterpenes displayed similar patterns in the data, with significant correlations between VOCs such as α-pinene and β-pinene ($R2 = 0.58$, $p = 1\times10\text{-}29$). Reproducibility was also assessed across the cohorts, which displayed that longitudinal VOC data was significantly more reproducible relative to cross-sectional ($p = 0.005$). Nevertheless, chemometric analyses could not distinguish the replicates of different volunteers, pointing toward a universal healthy breath baseline that needs to be verified in future studies with samples collected from subjects with diverse medical conditions.

Authors: Woollam M (1,2), Agarwal M (1,2,3)

Affiliations: 1. Integrated Nanosystems Development Institute, Indiana University Indianapolis, IN 46202, USA. 2. Department of Chemistry and Chemical Biology, Indiana University Indianapolis, IN 46202, USA. 3. Department of Biomedical Engineering and Health Informatics, Indiana University Indianapolis, IN 46202, USA

Michal Skawinski

Maastricht University

The in vitro effects of different bread types on fecal microbiome composition and volatile metabolic activity in healthy and non-coeliac wheat sensitive subjects

Michal was born and raised in Poland, where he studied Bioinformatics before moving to the Netherlands to dive deeper into Systems Biology. Now, he is working on his PhD at Maastricht University, focusing on volatolomics and metagenomics data analysis in the context of intestinal health. Science-wise, Michal is interested in how the human microbiome, nutrition, immunology, and mental health are all connected, and how we can use that knowledge for better diagnostics, profiling, and preventive treatments to improve well-being. Outside of science, Michal enjoys practicing yoga and experimenting with plant-based cooking.

Abstract:

The in vitro effects of different bread types on fecal microbiome composition and volatile metabolic activity in healthy and non-coeliac wheat sensitive subjects

Digestive diseases represent a substantial health concern, impacting the well-being and quality of life of affected people, with a global prevalence of approximately 30% in 2019, and rising. Non-Coeliac Wheat Sensitivity (NCWS) is a condition characterized by an adverse reaction to wheat consumption in the absence of coeliac disease or wheat allergy. The individuals affected by NCWS suffer from bloating, discomfort, flatulence, and abdominal pain, but extra-intestinal symptoms such as depression and anxiety are also present. The underlying etiological factors and mechanisms triggering this disease are currently not well understood. However, diet and consequently the intestinal microbiota composition and activity are considered to play an important role in symptom generation. Moreover, the occurrence of different symptoms has been previously associated with the grain content and the specific fermentation method, but their precise impact remains inadequately explored.

Aims:
The aim of the study was to investigate the impact of different breads on the in vitro composition of fecal microbiota and associated volatile organic compounds (VOCs) in NCWS individuals and healthy controls.

Methods:
Fecal samples from five NCWS and healthy controls 12 hours after bread consumption were incubated in vitro with different pre-digested breads, after dilution in standard SIEM medium in an anaerobic chamber. Breads made from wheat, emmer, or spelt either yeast or sourdough fermented were tested. At the baseline and after 5 hours of incubation fecal samples were taken using dedicated anaerobic culture vials, equipped with carbon sorption devices to collect VOCs. Microbial composition was profiled using amplicon sequencing of the hypervariable V4 region of the 16S rRNA gene. For VOCs analysis an additional measurement was taken after 2 hours of incubation. The fecal VOCs headspace were obtained using HiSorb with Gas Chromatography Time-of-flight Mass Spectrometry. Pre-processed data were analyzed using multivariate methods, and statistical importance was assessed with ANOVA Simultaneous Component Analysis.

Results:
Both microbiota and VOC profiles between NCWS and healthy controls showed distinct clustering of features primarily according to donors and time. Clear time shifts associated with exposure to bread were observed. However, the effects of the different bread types on the overall microbial community structure and metabolic activity were not pronounced.

Conclusions:
In conclusions, microbiome and VOC profiles analysis shows potential as a non-invasive monitoring tool for patients with NCWS symptoms. Validation of the results is needed in human subjects under controlled conditions to provide a robust foundation for the development of targeted dietary interventions tailored to NCWS individuals.

Authors and Affiliations
M. Skawinski 1, Prof. Dr Jonkers 2, Dr J. Penders 3, H. Becker 3, A. Mommers 1, Prof. Dr F.J. van Schooten 1, Dr A. Smolinska 1

1 Department of Toxicology and Pharmacology, NUTRIM Institute of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands
2 Department of Internal Medicine, Division of Gastroenterology and Hepatology, Maastricht University Medical Centre, Maastricht, The Netherlands
3 Department of Medical Microbiology, Infectious Diseases and Infection Prevention, NUTRIM Institute of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands

Standardization in Breath Analysis and Building a VOC Atlas

Arghavan Victoria Nawaby, Ph.D., MBA

US Food & Drug Administration

An Aim to Standardize Breath Analysis – Developing Regulatory Science Tools

BA.Sc. Chemical Engineering, University of Ottawa, CANADA (Cum Laude- 1991). M.A.Sc. Chemical Engineering, University of Ottawa, CANADA (1993). Chemical Engineer, Atomic Energy of Canada, CANADA (1993 – 1995), Ph.D. Physical Chemistry, Carleton University, CANADA (1999). Postdoctoral Fellow, Polymer Physics– NSERC Scholar, National Research Council of Canada, CANADA (1999 -2000). Associate Scientist– National Research Council of Canada, CANADA (2000 – 2007). JSP Fellow – Young Scientist Award, Tsukuba Science City, JAPAN (2006). Corporate Scientist – Sealed Air Corporation, USA (2007 – 2017). Principal & lead Consultant – AP Chem4 Solutions LLC, USA (2018 – 2021). Council Member - Gerson Lehrman Group (GLG), USA (2019 - 2021), Assistant Director – FDA, DBCMS, USA (2021 – Present). Executive MBA, University of Texas at Austin – McCombs School of Business, USA (May 2022).

Empathic and experienced thought leader with 27 years of knowledge in product development from infancy through commercialization. Working for organizations globally has given me the opportunity to effectively apply my scientific expertise and resolve technical challenges while building lasting relationships with my managers, peers, direct reports, and collaborators.

My passion for science and its application to solve exacting business issues gives me the capacity to constantly learn, self-improve, and apply my knowledge to effectively bridge business and science concepts for a more strategic and effective management approach (Winner of 2022 POETS & QUANTS – Best and Brightest Executive MBA, 2024 FDA Honor Award for External Partnership).

Abstract:

An Aim to Standardize Breath Analysis – Developing Regulatory Science Tools

Authors: Arghavan Victoria Nawaby, Michael Eppihimer, Dorn Carranza

United States Food & Drug Administration - FDA/CDRH/OSEL/DBCMS, Silver Spring, Maryland - USA

Geographic isolation and poverty are barriers to disease diagnosis in United States and many parts of the globe due to high cost and complexity of instruments needed to perform analysis. Supporting development of field instruments by having accurate databases of healthy breath and disease biomarkers is an important step towards an improved and equitable healthcare system[1]. Breath analysis is a promising technique that uses the biological information in exhaled breath to monitor, diagnose, and study human health while having the potential of decreasing turnaround times and providing real time analysis. A systematic approach in breath sample collection and biomarker confident identification can aid in establishing a web-based software tool to analyze mass spectrometry data. Development of relevant assessment approaches and Regulatory Science Tools (RST) can then be utilized by the in vitro diagnostics industry to help accelerate innovation and standardize identification of disease through breath [2,3].

Acknowledgements:
The authors would like to acknowledge the Bill & Melinda Gates Foundation for their support of this study.

References:
1. Ferrandino, G., et al. High-quality identification of volatile organic compounds (VOCs) originating from breath. Journal of Clinical and Translational Hepatology. 2023;11(3),638–648
2. Regulatory Science Tool Catalog - Catalog of Regulatory Science Tools to Help Assess New Medical Devices | FDA
3. FDA – OSEL - CDRH OSEL - Home (sharepoint.com)

Eric Miller, Ph.D.

US Food & Drug Administration

Confident Identification of Breath Matrix Compounds: An RST to Serve the in vitro Diagnostic Industry

Eric is an experienced analytical chemist and bioengineer with a breadth of industry experience that spans medical devices, natural products, and commercial laboratory testing. Having transitioned to the regulatory sector, he now applies these insights and perspectives to advance regulatory science in ways that foster innovation. As an FDA scientist, Eric's multidisciplinary background and international collaborative efforts are leveraged in dynamic projects with far-reaching implications. Through these collaborations, Eric derives a great deal of satisfaction knowing his research has a significant positive impact on public health equity and technology.

Abstract:

Confident Identification of Breath Matrix Compounds: An RST to Serve the in vitro Diagnostic Industry

Authors: Eric Miller, Ph.D., Samanthi Wickramasekara, Ph.D., Mark Annunziato, Ph.D., Jamie Kim, Ph.D., Krishna Patel, Ph.D., and Chathurika Rathnayake, Ph.D.

United States Food & Drug Administration - FDA/CDRH/OSEL/DBCMS, Silver Spring, Maryland - USA

Exhaled breath contains over 1,000 volatile organic compounds (VOCs) that can serve as biomarkers for various diseases, such as tuberculosis[1]. Until field-deployable diagnostic instruments are developed, various breath sampling methods are used to capture biomarkers at the patients' point of care (POC) for subsequent analysis at central labs. Once these biomarkers have been confidently identified among the complex matrix of other breath molecules, targeted analysis can be transferred to miniaturized field devices. Since biomarkers can be identified by comparing 'healthy' breath to 'disease' breath samples, it is crucial to first define the healthy human breath matrix—its compounds and concentrations. This provides a reference for detecting chemical differences in healthy vs diseases breath. However, confidently identifying chemicals in breath samples is a complex and a challenging process that lacks standardization[2]. This project will address these challenges by utilizing the ReCIVA® Breath Sampler for sample collection and HRMAS GC-MS for analysis to build a detailed database of breath chemical information, establish criteria for reliable VOC identification, and develop web-based software for analyzing mass spectrometry data related to breath samples[3].

Acknowledgements:
The authors would like to acknowledge the Bill & Melinda Gates Foundation for their support of this study.

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1. Phillips M., et al. Breath biomarkers of active pulmonary tuberculosis. Tuberculosis. 2010;90,145-151.
2. Henderson B., et al. A benchmarking protocol for breath analysis: the peppermint experiment. Journal of Breath Research. 2020; 14, 046008.
3. Ferrandino, G., et al. High-quality identification of volatile organic compounds (VOCs) originating from breath. Journal of Clinical and Translational Hepatology. 2023;11(3),638–648.

Dr Omar E. Rivera

US Food & Drug Administration

Evaluating Method Performance for Breath Analysis

Dr Omar E. Rivera started working at the U.S. Food and Drug Administration in March 2023. His research currently focuses on identifying and characterizing extractables and leachables. Currently involved on developing ways of evaluating method performance for breath analysis. Dr Rivera's prior employment history has been working for the federal government as a contractor. For three years he worked with U.S. Customs and Border Protection aiding officers from out in the field identify illicit narcotics substances that were smuggled to the country through different spectroscopic and chromatographic techniques. Started his career working at the Savannah River National Laboratory (SRNL) for the Non-proliferation technologies sections for five years. The group developed a series of devices that were tested in areas of suspected illegal weapons development.

Abstract:

Evaluating Method Performance for Breath Analysis

Authors: Omar Rivera Betancourt, Joshua A. Young, Nimesh P.R. Ranasinghe Arachchige, Sadeepa Mallikarachchi, Kanti Sapkota
United States Food & Drug Administration - FDA/CDRH/OSEL/DBCMS, Silver Spring, Maryland -USA

Abstract
Confident, efficient, and reliable detection of TB biomarkers in patient's breath is a path to disease detection and treatment to promote human health equity and equality. This project will develop a framework to ensure that breath researchers globally will have the tools to develop analytical methods such that the resulting data can be trusted and compared. The goal will be to incorporate established models[1-3] into a chemical space to determine what would be detectable in samples. We will also establish method performance parameters for expanding detectability of specific regions within the chemical space. This will be achieved via a scoring system that ensures robustness of analytical methods for chemical detection from breath matrix. The study will also provide a coverage map for breath VOCs, which will allow users to access information on appropriate analytical methodologies for specific VOCs of interest. Lack of guidance on selection of breath reference standards, and a lack of method criteria for the evaluation of data quality for reliably detecting and identifying breath biomarkers was the motivation of the research identified by OSEL/DBCMS and the Bill and Melinda Gates Foundation.

Acknowledgements:
The authors would like to acknowledge the Bill & Melinda Gates Foundation for their support of this study.

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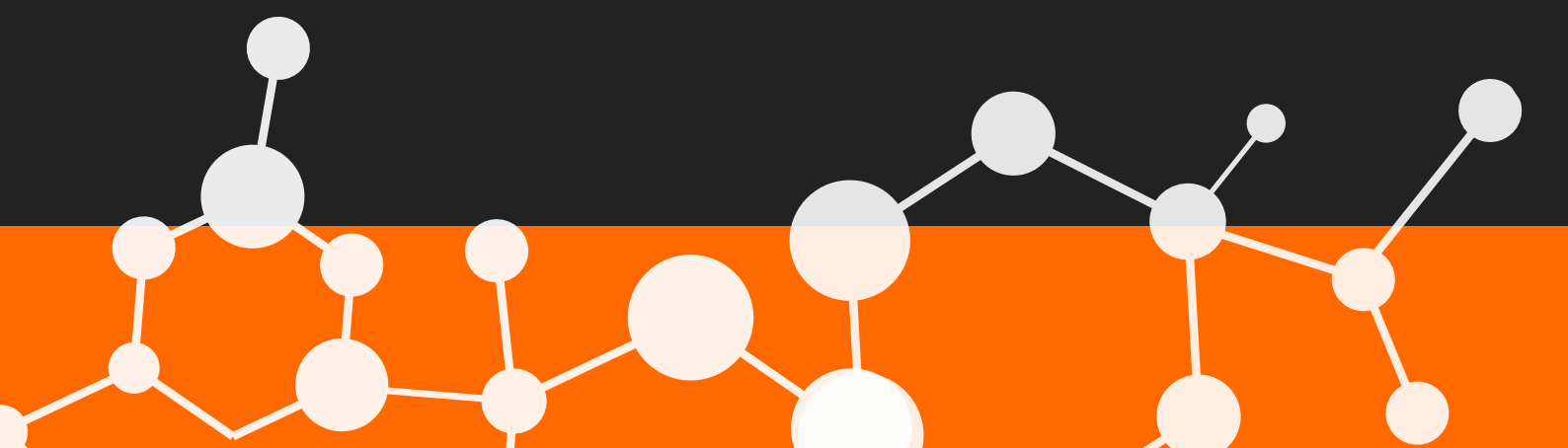
1. Bose, A., Westmoreland, P.R., J. Phys. Chem. A 2020, 124, 10600−10615.
2. Black G. et al. Analytical and Bioanalytical Chemistry 2023, 415, 35–44.
3. Rapp, D.; Englander-Golden, P., J. Chem. Phys. 1965, 43, 1464– 1479.



Kavita M. Jeerage, PhD

National Institute of Standards & Technology (NIST)

Potential Uses of Breath Surrogates for the Development and Deployment of New Infectious Disease Breathalyzers

Dr Kavita M. Jeerage, PhD, leads efforts to develop and deploy standards for breath analysis, including both clinical analysis and forensic analysis, focused on cannabis breathalyzers. Dr Jeerage received degrees in chemical engineering from the University of Minnesota and the University of Washington, and joined NIST in 2006 through a National Research Council Postdoctoral Fellowship. She has a broad background in instrumental analysis, electrochemical sensors and actuators, material-fluid interactions, and in vitro toxicology. Starting in 2020, Dr Jeerage led the expansion of NIST's cannabis breathalyzer program into human studies and began creating NIST's clinical breath standards program. She is currently the principal investigator of two ongoing human studies that examine breath matrices for cannabis compounds and the feasibility of a two timepoint breath test to determine recent cannabis use.
She is also leading an effort to develop breath surrogates to benchmark breathalyzers for infectious disease. Dr Jeerage has partnered with researchers in public health and psychology to accomplish the goals of these programs and leads teams that include chemists, physicists, engineers, statisticians, theorists, and experimentalists.

Abstract:

Potential Uses of Breath Surrogates for the Development and Deployment of New Infectious Disease Breathalyzers

Malaria and tuberculosis, two infectious diseases that cause tremendous loss of life, are most reliably diagnosed via slow and/or expensive methods, such as microscopic examination of blood smears or cultured sputum samples, or by nucleic acids. Non-invasive identification via inexpensive sensors that target volatile organic compound (VOC) signatures in breath would provide an important point-of-care improvement. Sensors under development for this purpose can be benchmarked and eventually validated through the delivery of reproducible, matrix-matched breath surrogates. Such surrogates must contain compounds relevant to the disease(s) in question, at relevant concentrations, and must also mimic other relevant characteristics of human breath (e.g., temperature, humidity, carbon dioxide and oxygen content), and perhaps potential interferents that may be found in the breath of patients without the disease.

This presentation briefly summarizes potential VOCs of interest for the diagnosis of malaria and tuberculosis as identified through published studies of in vitro cultures, in vivo animal models, and humans exposed to these diseases (naturally or in controlled settings). This presentation also discusses existing approaches to delivering matrix-matched breath surrogates, and the system under development in our labs, which utilizes standard gas mixtures prepared in nitrogen. Approaches to address stability challenges in the cylinder or in the delivery system are discussed. In combination with the delivery system, standard gas mixtures will permit prototype devices to be evaluated in a controlled laboratory setting with known uncertainty for each component of the matrix-matched surrogate.

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(2) Chemical Sciences Division, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA 20899

Julia Greenwood

Owlstone Medical

Volatile Biomarker Discovery and the VOC Atlas

Julia joined Owlstone in July 2018 as a product development lead, and is currently the VP of Programme - Research Products and Services. She is responsible for our product strategy as well as managing our delivery teams. Julia has come from a background in product development: her previous roles include Principal Physicist at Cambridge Consultants where she worked on a wide range of technologies for consumer and industrial applications. Her primary focus was on sensing technologies and optical physics. Julia studied Physics at the university of Bristol and went on to do research in Surgical Robotics and Medical Imaging at Imperial College London.

Abstract:

Volatile Biomarker Discovery and the VOC Atlas

Volatile organic compounds (VOCs) can arise from underlying metabolism and are detectable in exhaled breath, therefore offering a promising route to non-invasive diagnostics. Owlstone Medical has undertaken a large project to build a VOC Atlas as a reference database of chemically confirmed VOC identities that are genuinely breath-borne. This will facilitate future breath biomarker discovery and subsequent biomarker validation in clinical studies and can be used to facilitate cross-study data comparisons for improved standardization in the field of breath research. Building the VOC Atlas requires precise, accurate, and robust workflows for quantification and chemical identification. The atlas project is also being expanded and made public with support from key collaborative partners such as the FDA, Gates Foundations, and FDA to provide the highest-quality resource to support breath as a biomarker platform for clinical and research use.

Ask the Expert Panel Discussion

Standardization in Breath Analysis and Building a VOC Atlas

Meet the Panelists:

In our always popular "Ask the Expert" panel discussion, we bring together world leaders in breath research to tackle challenges, discuss future directions, and establish best practices within the field. Every year, we choose a topical focus and gather relevant experts to answer questions around this theme, allowing time fordebate and discussion around the answers with other experts.

In 2024, our Ask the Expert session will center on achieving better standardization in breath research and building a VOC atlas. Chaired by our CEO and Co-Founder Billy Boyle, this discussionwill explore innovative strategies and analytical approaches to enhance standardization within the field.

James Covington
Professor in Electronic Engineering, University of Warwick

Iain White
Assistant Professor, University of Manchester

Billy Boyle
CEO and Co-Founder, Owlstone Medical

Brooke Kaiser
Principal Investigator, Pacific Northwest National Laboratory

Ben de Lacy Costello
Associate Professor Biosensing and Diagnostics, University of Bristol

Posters

Posters: *Click the title to visit the poster*

AI-Driven Exhalation Biopsy Technology for the Detection of Gastrointestinal Cancer
Presented by Li Shangzhewen, Fudan University

Breath Analysis: Identification Of Potential Volatile Biomarkers For Non-invasive Diagnosis Of Chronic Kidney Disease (Ckd)
Presented by Alessia Di Gilio, University of Bari

Breath Biopsy® Reveals Promising Voc Biomarkers For Early Detection And Monitoring Of Acute Pulmonary Exacerbations In People With Cystic Fibrosis
Presented by Lucy Gale, University of Cambridge

Development and Validation of a Portable Device for At-Home Hydrogen and Methane Breath Testing
Presented by Rui Lopes, Owlstone Medical

Exploring exhaled breath volatile organic compounds in occupational asthma: A pilot cross-sectional study
Presented by Bato Hammarström, Oslo University Hospital

Food And Lifestyle Impact On The Exhaled Breath Vocs In Professional Athletes
Presented by Milena Aleksić, BioSense Institute, University of Novi Sad

GYNE-VOX: Clinical study for the analysis of volatile organic compound profiles for endometrial cancer screening
Presented by Bernado Raimundo, Champalimaud Centre for the Unknown

High sensitivity detection of deuterium-labelled volatile compound probes with Orbitrap-MS: from in-vitro headspace to Breath Biopsy for lung cancer
Presented by Alexandra Martin, Owlstone Medical

HiSorb TD-GC-MS Innovation: Probing Liver Metabolism with Deuterated EVOCs Unveils Potential Novel Enzymatic Pathways and Biomarkers for Disease Diagnosis
Presented by Antonio Murgia, Owlstone Medical

Posters: *Click the title to visit the poster*

Maintaining sample integrity in breathomics workflows with TD-GC-TOF- MS
Markes International and SepSolve Analytical Sponsored Poster

Real-Time Analysis of Exhaled Breath Using SIFT-MS for Biomarker & Disease Identification
Syft Sponsored Poster

Strategy for Enabling Clinical Confidence in Breath Analysis
Presented by Fred Farrow-Dunn, The National Physical Laboratory

Systemic Sclerosis: Can Breathomics help clinicians for ILD management?
Presented by Thibault Massenet, University of Liège

Targeted Breath Biopsy® Profiling Of Induced Biomarkers Unveils A Metabolic Adaptation In Cirrhosis Toward Alcohol Production
Presented by Giuseppe Ferrandino, Owlstone Medical

The Breath Analysis For Early Detection Of Malignant Pleural Mesothelioma (Mpm) and Management Of Asbestos Exposure Subjects
Presented by Marirosa Rosaria Nisi, University of Bari

Tunable Diode Laser High-Resolution Spectroscopic Measurements of the Microbiota Gaseous Biomarkers Kinetics
Presented by Oleg Medvedev, Moscow State University

Validation Methane And Hydrogen Portable Breath Analyzer Aire2 Through Calibration Gases
Presented by Nikita Fadeev, Moscos State Technical University

Volatile Organic Compounds (Vocs) In The Exhaled Breath As Biomarkers For The Early Detection Of Lung Cancer: Application Of Complementary Methodological Approaches
Presented by Jolanda Palmisani, University of Bari

Owlstone Medical

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