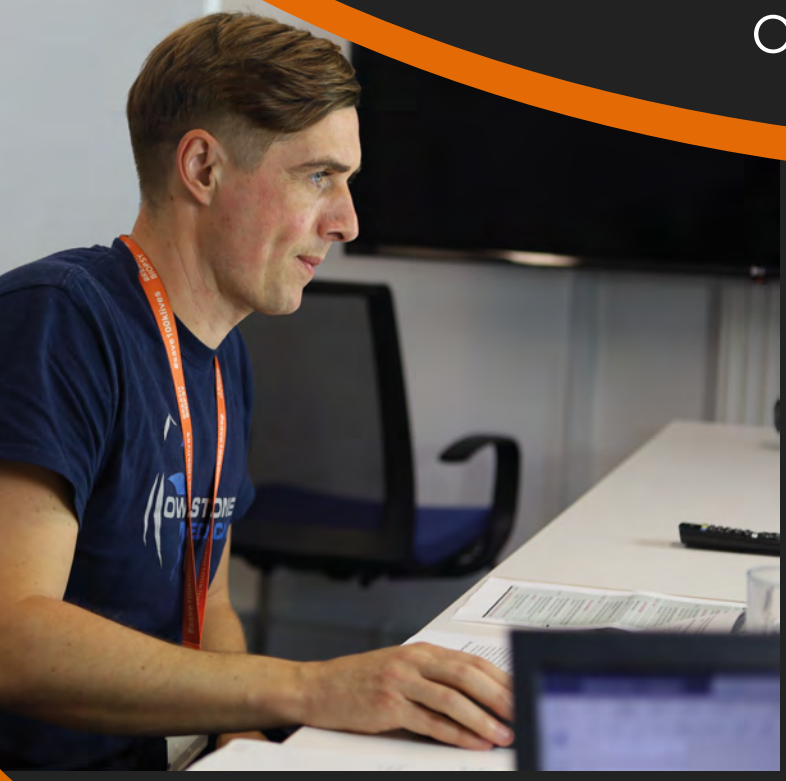
BREATH® BIOPSY

Breath Biopsy Conference

31st October – 1st November 2023

Online



Day One

Plenary Speaker

Michael Wilde

University of Plymouth

10:00am - 11:00am	Plenary Talk with Michael Wilde
	'Evidence of Breath Metabolite Networks'
11:00am - 11:15am	Sponsored Talk with Thermo Fisher
Tilly Woodland	*'Precision Methods and Higher Throughput Workflows using Thermo Scientific Chromeleon 7.3.2 Software for VOC Atlas Building'*
11:15am - 12:00pm	Poster Session
	LUNCH BREAK
1:00pm - 2:45pm	Advances in Technologies ***Including talks from***
Boehringer Ingelheim	*'Development of a Novel Method for Analysing Volatile Organic Compounds from Intubated Mice'*
Robert Rintoul	*'Progress in the development of a Breath Biopsy test for the early detection of lung cancer; The LuCID and Evolution studies'*
2:45pm - 3:00pm	Sponsored Talk with Markes International and SepSolve Analytical
Laura Miles	*'From Research to Routine Screening: Meeting the Evolving Demands of Clinical Breath Analysis with Thermal Desorption'*
3:00pm - 4:00pm	Early Careers Talks ***Including talks from***
Kathleen Zwijsen - University Antwerp	*'Using Ion Mobility Spectrometry for Early Mesothelioma Detection in Asbestos-Exposed Individuals: an External Validation'*
Yuta Matsuoka - Kyoto University	*'Breath biopsy technology targeting for ferroptosis-related volatile metabolites.'*
Iris van der Sar - Erasmus University Medical Center	*'eNose Technology in Interstitial Lung Disease'*

Day Two

Plenary Speaker

Sean Harshman

Air Force Research Laboratory,
UES Inc

10:00am - 10:30am	Gastrointestinal and Microbiome Extended Session ***Including talks from***
Giuseppe Ferrandino - Owlstone Medical	*'Gut Microbiota Profiling Via Dynamic Breath Analysis'*
10:30am - 12:00pm	Applications of Breath Analysis ***Including talks from***
Pedro Vaz - Champalimaud Cancer Centre	*'A Pragmatic Approach For Lung Cancer Screening Using Exhaled Breath In A Clinical Setting'*
Herbert Fink - Roche	*'Indole as a new tentative marker in exhaled breath for non-invasive blood glucose monitoring of diabetic subjects'*
Luis Mendez - Clinica Alemana	*'Approaching Complementary Studies for Timely and Accurate Diagnosis of Cirrhosis. From Primary Care to Specialty Consultation'.*
12:00pm - 12:30pm	Poster Session
LUNCH BREAK	
1:30pm - 2:30pm	Plenary Talk with Sean Harshman
'Impact of Thermal Desorption Tubes on the Variability of Exhaled Breath'	
2:30pm - 3:30pm	Applications of Breath Analysis ***Including talks from***
Bruce Johnson - Mayo Clinic	*Volatile Organic Compounds in Exhaled Breath Reflect Physiological Changes in Ultramarathon Runners*
Moniek van Aarle - Maastricht University Medical Centre	*Exhaled Breath Analysis for Asthma Detection in Children: Leveraging and comparing SIFT-MS and GC-ToF-MS*
SHORT BREAK AND QUESTIONS FOR THOUGHT	
4:00pm - 5:00pm	Ask the Expert - Panel Discussion See the Experts in the Pages Below

Dr Michael Wilde

University of Plymouth

‘Evidence of Breath Metabolite Networks’

Meet Michael -

Dr Michael Wilde is a lecturer in Analytical & Environmental Chemistry at the University of Plymouth. He is also a recent recipient of a CAMS UK Lectureship award in Bioanalytical Measurement and Volatilomics from the Community of Analytical Measurement Science, and he’s been settling in at Plymouth by acquiring state-of-the-art equipment for the analysis of biological and environmental samples.

Before his appointment at Plymouth, Mike was previously a research fellow at the University of Leicester, where his research contributions were significant in the MRC Molecular Pathology Node, called EMBER becoming recognized as an internationally leading centre in breath research. By developing cutting-edge analytical technologies for the measurement of volatile metabolites in breath, working between the hospital and the lab, Mike was central in delivering over 2000 patient breath/VOC samples, integrating advanced chromatographic and mass spectrometric technologies in over 5 clinical studies across respiratory and cardiovascular science.

His research portfolio reflects a passion for discovery, the separation of complex mixtures, and advancing the application of multidimensional gas chromatography-mass spectrometry. Consequently, he has a continued interest in the improvement of chemometric workflows, and alongside breath, his research interests include volatile metabolites in vitro, environmental health such as air quality, and microplastics.

Plenary Talk

Abstract:

Acute breathlessness due to cardio-respiratory diseases accounts for more than 1 in 8 of all emergency admissions to hospital [1]. Diagnostic evaluation based on blood-based biomarkers and radiological tests is difficult in patients with multifactorial presentations of acute breathlessness and particularly challenging to interpret in the context of pre-admission treatment exposure [2]. The assessment of exhaled, low-molecular weight metabolites, chemically classified as volatile organic compounds (VOCs), offers new opportunities for the development of rapid, non-invasive diagnostic and prognostic biomarkers.

As part of the East Midlands Breathomics Pathology Node (EMBER), breath VOCs were sampled from acutely unwell hospitalised patients presenting with breathlessness due to severe exacerbations of asthma, chronic obstructive pulmonary disease, heart failure or pneumonia and matched healthy controls (n=277) [3]. By isolating and visualizing exhaled VOCs with multidimensional gas chromatography-mass spectrometry (GC×GC-MS), coupled with rigorous clinical phenotyping, exhaled breath metabolites were shown to have high diagnostic accuracy (79% sensitivity, 85% specificity) for severe cardiorespiratory exacerbations, including in the presence of clinical uncertainty [4].

The discovery of significantly enriched metabolite sets with high chemical similarity revealed new insights into the dysregulation of breath metabolite networks across several pertinent volatile classes in different clinical subtypes of cardiorespiratory exacerbation. Research from the EMBER Node demonstrates how breath biomarker platforms may be used in acute care and the potential for the translation of this technology into a real-world clinical setting.

References:

[1] Hutchinson, A., et al., Breathlessness and presentation to the emergency department: a survey and clinical record review. BMC pulmonary medicine, 2017. 17(1): p. 53-53.
[2] Parshall, M.B., et al., An Official American Thoracic Society Statement: Update on the Mechanisms, Assessment, and Management of Dyspnea. American Journal of Respiratory and Critical Care Medicine, 2012. 185(4): p. 435-452.
[3] Ibrahim, W., et al., Assessment of breath volatile organic compounds in acute cardiorespiratory breathlessness: a protocol describing a prospective real-world observational study. BMJ open, 2019. 9(3): p. e025486.
[4] Ibrahim, W., Wilde, M. and Cordell, C., et al., Visualization of exhaled breath metabolites reveals distinct diagnostic signatures for acute cardiorespiratory breathlessness. Science Translational Medicine, 2022. 14(671): p. eabl5849.

Dr Sean Harshman

US Air Force

‘Impact of Thermal Desorption Tubes on the Variability of Exhaled Breath’

Meet Sean -

Dr. Sean Harshman is a Research Scientist and the Analytical Chemistry Team Lead in the Biotechnology Branch within the Airman Biosciences Division of the 711th Human Performance Wing (711HPW/RHBBA). Dr. Harshman leads a diverse team of scientists and engineers supporting the mission of the Air Force through research and core functionality utilizing mass spectrometry and biochemical analyses. His research focuses on biomarker discovery utilizing non-invasive or minimally invasive biosources to predict the physiological state of Airmen and Guardians. Dr. Harshman has mentored over a dozen high school, undergraduate, and graduate students and post-docs within the lab. He holds adjunct status at Wright State University providing lectures on proteomics within the Systems Biology course of the Biochemistry Department. Dr. Harshman is on the editorial board of the Journal of Breath Research and is a standing member of the International Association for Breath Research and the American Society for Mass Spectrometry.

Following his graduate studies at The Ohio State University, Dr. Harshman joined the Air Force as a contract researcher, in the labs of Dr. Curt Grigsby and Dr. Jennifer Martin, leading the contract personnel supporting biomarker discovery efforts within the 711th Human Performance Wing. He ultimately transitioned to his current position as a government civilian, as the Analytical Chemistry Team Lead, n 2021.

Plenary Talk

Abstract:

Due to the overall low abundance of volatile compounds in exhaled breath, preconcentration of the sample prior to traditional thermal desorption gas chromatography mass spectrometry (TD-GC-MS) analysis is be necessary. While certain aspects of thermal desorption tubes, such as volatile storage, have been evaluated, many aspects remain uncharacterized. Two common thermal desorption tubes, Tenax TA and Biomonitoring 5TD tubes, were evaluated for background content and flow rate variability. The data illustrate that the Biomonitoring 5TD tubes have the highest number (23) and abundance of background contamination greater than 3x the noise when compared to Tenax TA (13) and empty tubes (9). Tentative identifications of the compounds in the background contamination experiment show that greater than 59% (16/27) of the compounds identified have been reported in the breath literature. Flow rate measurements of 200 Tenax TA and 200 Biomonitoring 5TD tubes show a large range in measured flow rates among the TD tubes (Tenax: 252.9-284.0mL min-1, 5TD: 220.6-255.1mL min-1). Tenax TA and Biomonitoring 5TD tubes which had high, medium, and low flow rates were tested with gas standards and an exhaled breath peppermint experiment. These results show insignificant differences ($p>0.05$) among the tubes of different flow rates. While measured flow rate variability is present, the data demonstrate no statistically significant difference was observed between tubes showing high, medium, and low flow rates.

Tilly Woodland

Owlstone Medical

on behalf of

Thermo Fisher Scientific

‘Precision Methods and Higher Throughput Workflows using Thermo Scientific Chromeleon 7.3.2 Software for VOC Atlas Building’

Meet Tilly-

Tilly Woodland is a Senior Analytical R&D Scientist at Owlstone Medical, where she has worked for 4 years. Her academic background is in Analytical Chemistry, which she now uses to jointly lead the team responsible for developing the new targeted and untargeted method within Owlstone Medical's Research Products and Services programme.

Sponsored By

ThermoFisher SCIENTIFIC

Abstract:

In the pursuit of constructing a quantitative Atlas of volatile organic compounds, through the analysis of customer studies, the pivotal components of precision methods and efficient data processing workflows are essential. This talk explores the development of new methodologies that facilitate the development of a comprehensive VOC Atlas, with an insight into the development of Owlstone's new targeted/untargeted method and the utilisation of the Chromeleon v7.3.2 software upgrade. This upgraded software exhibits the capacity to process complex targeted panels with improved speed and efficiency.

The synergy between the new method and the upgraded Chromeleon software not only accelerates data processing but also ensures data quality, contributing to the construction of a more reliable and comprehensive Atlas. This presentation underscores the importance of these elements in Atlas building, offering practical insights used within OML for achieving improved analytical workflows.

Laura Miles

Markes International

‘From Research to Routine Screening: Meeting the Evolving Demands of Clinical Breath Analysis with Thermal Desorption.’

Meet Laura -

As in the thermal desorption business unit Laura is responsible for developing new methods and testing Markes International’s suite of thermal desorbers for new and emerging applications. Laura joined Markes International as a customer support specialist in 2014 before moving to work in application development. As part of her current role Laura works closely with key opinion leaders in collaborations across a variety of market areas and she has a particular specialism in environmental analysis, breathomics and defence and forensics.

Sponsored By

MARKES international

SepSolve Analytical

Abstract

Breath based biomarker discovery research is entering a particularly exciting phase with many studies scaling up to large clinical trials and generating viable candidate marker compounds. As the size of sample sets increases so does the length of individual studies and the number of personnel involved. This scale-up of efforts introduces more opportunities for error and highlights the need for rigorous quality control.

Thermal desorption (TD) coupled with GC-MS has long been established as the gold standard technique for breath analysis. Volatile organic compounds (VOCs) in breath are pre-concentrated by collection onto sorbent packed tubes and shipped to a central laboratory for analysis. These tubes are small, physically robust, easy to transport and provide extended sample storage stability compared to other methodologies.

In this presentation we will discuss how quality control strategies can be built into thermal desorption-based workflows throughout the life cycle of every sample tube. These strategies include the use of surrogates and internal standards, automated water management, sample archiving and extending dynamic ranges with automated split re-collection. We will also explore the generation of pooled biological quality control samples from breath and how they could be applied to longitudinal studies in evaluating the quality of the data, reproducibility of the method and instrumentation, as well as correcting for any system bias over time.

The drive for standardisation across breathomics workflows is essential to support the transition from research into routine screening and the future evolution of clinical breath analysis.

Authors

Laura Miles[1], Helen Martin[1] and Ryan Francis[1]

Affiliations

1 Markes International Ltd, 1000B Central Park, Western Avenue, Bridgend, CF31 3RT, UK

Dr Luis Mendez

Clinica Alemana

‘Approaching Complementary Studies for Timely and Accurate Diagnosis of Cirrhosis. From Primary Care to Specialty Consultation.’

Abstract:

Cirrhosis is a growing global health problem. Its prevalence and the pressure on different health systems are expected to increase. Early detection of cirrhosis is a challenge due to the limitations of clinical tools and complementary methods in terms of accuracy, cost or availability.

The study of the volatiloma offers new possibilities for a non-invasive, reproducible diagnosis that could also be available in primary care. Extensive prospective validation is required to establish its definitive role.

The correct combined use of clinical skills in combination with complementary studies is possibly the best approach today for an efficient approach to the diagnostic challenge of cirrhosis diagnosis of cirrhosis. For an efficient confrontation of the diagnostic challenge of cirrhosis It is essential that clinicians can be trained in the proper use of each test in order to optimise their performance.

Dr Pedro Vaz

Champalimaud Cancer Centre

‘A Pragmatic Approach For Lung Cancer Screening Using Exhaled Breath In A Clinical Setting.’

Meet Pedro -

Pedro graduated in Chemistry with specialization in organic chemistry. Still as undergraduate he worked in the USA under supervision of Prof. Rudolph Abramovitch (Clemson University, USA), on a NATO sponsored project. A PhD in Chemistry at the University of Aveiro (Portugal) followed focusing on structural characterization (vibrational and neutron spectroscopy, mass spectrometry and quantum simulations) and intermolecular interactions in dynamical systems. In the 2008-2015 period, he held positions as a staff researcher at the University of Lisbon, Portugal and at the ISIS Neutron & Muon Facility, UK. In 2015 he moved into the private sector serving as scientific director at SGS Portugal, an inspection and quality assurance multinational. In parallel, he is a consultant for companies across different sectors, such as phytopharmaceuticals (Ascenza, Portugal), precision machining (Argobaum, Portugal) and lab design and construction (ERT, Portugal), which are still ongoing. In 2018 Pedro joined Champalimaud Foundation as researcher at the Lung Unit where he is leading the development of early diagnostics

for lung cancer using exhaled breath analysis. From 2020 onwards, he is head scientist at Tellspec Inc. (Canada/UK), implementing and validating spectroscopy solutions for non-invasive chemical analysis with applications in the health sector. Pedro gave invited to lecture at UPMC, Sorbonne (Paris, France), Osaka Prefecture University (Osaka, Japan) and King Abdullah University of Science & Technology (Thuwal, Saudi Arabia). Since 2019 he is an elected fellow of the Royal Society of Chemistry, UK.

Abstract:
Lung cancer is hardly detected at early stages. The rate for lung cancer detection at stage I is as low as 25%, which finds a correlation with the high number of deaths in patients with lung cancer as most cases are detected at later stages with much lower survival rates. For those with stage I disease, the chance of cure may reach 70%. As such, every methodology that can contribute to assisting in the identification/screening of lung cancer cases at an early stage must be a mandatory goal when developing screening programs. This is key as the incidence is forecasted to increase by 70+% by 2040 compared to 2020, according to GloboCan data.
Although established protocols for screening are set firefighting against cancer should not be the core aim of our efforts. Instead, Early Detection and Diagnosis (ED&D) must be the priority tool at the earliest clinically onset yielding much better chance of cure and survival with a concomitantly improved quality of life to patients. With this ambition, new and innovative approaches to screen lung cancer through, of which analysis of volatile organic compounds (VOC's) in exhaled breath air is promising. From the set of compounds, a few are key components, denoted biomarkers, but that has proven hard to attain. A more pragmatic approach adopted at Champalimaud Foundation looks at the complete profiles, with rewarding results so far and an ED&D tool starts to be envisaged.

Dr Bruce Johnson

Mayo Clinic

‘Breath Volatile Organic Compound Analysis Reveals Physiological Shifts in Ultramarathon Runners.’

Meet Bruce -

Bruce Johnson is a Professor of Medicine and Physiology at Mayo Clinic and Directs the Human Integrative and Environmental Physiology Laboratory as well as the institutional Energy Balance Core Laboratory. He pursues studies in clinical, environmental and human performance physiology with a general interest in the limits of human performance and adaptation to unique environments.

Abstract:

Exhaustive exercise can induce lung impairment and various physiological reactions in the human body. Volatile organic compounds (VOCs) are exchanged between breath and circulating blood in the lung alveoli, making them ideal non-invasive biomarkers for better understanding the effects of exhaustive exercise on the lungs and other parts of the body. Currently, only a few studies have utilized breath VOCs to investigate the effects of exhaustive exercise on physiological changes, and no research has explored breath VOC changes in the context of running an ultramarathon, which takes roughly 40 hours to complete.

To identify potential breath biomarkers of exhaustive exercise, we collected breath samples from 24 participants in the 2019 Ultra-Trail du Mont Blanc (UTMB) ultra-marathon before and after the race. All 48 samples underwent analysis using the Breath Biopsy Platform included with the Thermo Fisher GC-Orbitrap™ via TD-GC-MS. The Wilcoxon signed rank test, followed by the Benjamini– Hochberg False Discovery Rate ($p < 0.05$) was used to determine whether the breath VOC abundances differed before and after the race. Of the 793 VOCs identified in the breath samples, 63 showed significant differences between pre- and post-race samples (12 decreased, 51 increased). Tentatively identified VOCs indicate the involvement of fatty acid oxidation, inflammation, and possible altered gut microbiome activity in response to exhaustive exercise. These findings suggest significant changes in VOC abundance as a result of exhaustive exercise. Further investigations incorporating diverse physiological measurements are crucial for understanding the VOC biological associations in exhaustive exercise.

Authors: Hsuan Chou1, Kayleigh Arthur[1], Elen Shaw[1], Chad Schaber[1], Billy Boyle[1], Max Allsworth[1], Eli F. Kelley[2], Glenn M. Stewart[2,3], Courtney M. Wheatley[4], Jesse Schwartz[2], Catlin C. Fermoyle[2,5], Briana L. Ziegler[2], Kay A. Johnson[2], Paul Robach[6], Patrick Basset[7], Bruce D. Johnson[2]

[1]Owlstone Medical, Cambridge, UK; [2]Department of Cardiovascular Diseases, Mayo Clinic, Rochester, USA; [3]Menzies Health Institute Queensland, Griffith University, Brisbane, AUS; [4]Department of Cardiovascular Diseases, Mayo Clinic, Scottsdale, USA; [5]Utah Vascular Research Laboratory, Salt Lake City, USA; [6]Ecole Nationale des Sports de Montagne, Chamonix, FR; [7]Ultra Sports Science Foundation, Grenoble, FR.

Dr. Herbert Fink

Roche Diabetes Care

‘Indole as a new tentative marker in exhaled breath for non-invasive blood glucose monitoring of diabetic subjects.’

Meet Herbert -

Dr. Herbert Fink is a research scientist at Roche Diabetes Care, working within Development in the group of Chemical and Instrumental Analytics. Dr. Fink holds a PhD in Analytical Chemistry and joined Roche in 2008. His interests include all kinds of spectroscopy and since 2018 breath analysis.

Abstract:

Blood glucose monitoring is important in diabetes management but usually involves invasive finger pricking. A clinical study is reported using a non-invasive approach based on exhaled breath. Main objective was the discovery of volatile markers for prediction of blood glucose levels in diabetic patients.

Exhaled breath was sampled every 15 minutes in 60 diabetic patients (30 type 1, 30 type 2) for a period of 6 hours including a meal event. Proton-Transfer-Reaction-Time-of-Flight-Mass-Spectrometry (PTR-ToF-MS) was used to sample breath in real-time. Blood glucose was tested in parallel via test strips.

Exhaled indole (a bacterial metabolite of tryptophan) showed significant correlation to glucose. The type of diabetes did not affect this result. As indole has been linked to human glucose metabolism, it might be a tentative marker in breath for non-invasive glucose monitoring.

Authors:
Herbert Fink, Tim Maihöfer, Jeffrey Bender, Jochen Schulat

Reference:
J. Breath Res. 16 (2022) 026001 https://doi.org/10.1088/1752-7163/ac4610

Dr. Moniek van Aarle

Maastricht University Medical Centre

‘Exhaled Breath Analysis for Asthma Detection in Children: Leveraging and comparing SIFT-MS and GC-ToF-MS’

Abstract:

Asthma is a common chronic condition in childhood, with a high misdiagnosis rate. Current diagnostic algorithms for asthma are complex, and there is a need for non-invasive, reliable tests. Volatile organic compounds (VOCs) in exhaled breath have shown promise as potential biomarkers for asthma. However, the lack of a standardized breath test hinders its clinical implementation. This study aims at comparing the diagnostic accuracy of two breath analysis techniques, ‘Selected Ion Flow Tube Mass Spectrometry’ (SIFT-MS) and ‘Gas Chromatography Time of Flight Mass Spectrometry’ (GC-ToF-MS), for distinguishing between healthy and doctor-diagnosed asthmatic school-aged children.

The analytical observational cross-sectional study presented here includes 51 asthmatic school-aged children and 68 healthy controls. Breath samples were collected using a tailored and child-friendly sampling system. SIFT-MS and GC-ToF-MS were used to analyse the exhaled breath samples. Principal component analysis (PCA) and unsupervised random forest models were employed for exploratory data analysis. Classification

models were built to evaluate the discrimination power of different VOCs detected by both techniques. The analysis of exhaled breath samples using SIFT-MS and GC-ToF-MS revealed a diverse set of volatile organic compounds (VOCs). While the PCA scores plots did not show clear groupings, the classification models based on discriminatory VOCs exhibited promising sensitivity and specificity values. The build classification model for SIFT-MS identified 47 discriminatory variables and achieved a sensitivity and specificity of both 70%. The GC-ToF-MS analysis identified 17 discriminatory variables and demonstrated a sensitivity and specificity of 76% and 70%, respectively. The combination of both techniques might improve the diagnostic model even further. Our study highlighted the potential of SIFT-MS as a user-friendly and reliable breath diagnostic tool for paediatric asthma. The discriminatory VOCs identified by SIFT-MS and its comparable diagnostic accuracy to GC-ToF-MS underscore its feasibility for real-world clinical implementation. This brings us closer to developing a quick and accessible breath test for asthma diagnosis in children, enabling the utilisation of precision medicine in asthma management. We acknowledge, though, the potential influence of inhaled corticosteroid (ICS) medication on breath VOC profiles of asthmatic participants. To address this limitation, future studies should specifically explore the effect of ICS on VOC patterns to ensure accurate diagnostic performance. In conclusion, SIFT-MS holds promise as an effective and user-friendly alternative to GC-ToF-MS, advancing breath diagnosis towards practical clinical application in paediatric asthma.

Authors: Kienhorst S (1)* & van Aarle MHD (1)* Vitale R (2) Smolinska A (3)# and Dompeling E (1)# *shared first author #shared last author

Affiliations: (1) Department of Paediatric Pulmonology, Maastricht University Medical Centre, Maastricht, The Netherlands (2) U. Lille, CNRS, LASIRE, Laboratoire Avancé de Spectroscopie pour les Interactions, la Réactivité et l'Environnement, Cité Scientifique, F-59000 Lille, France (3) Department Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

Dr. Giuseppe Ferrandino

Owlstone Medical

‘Gut Microbiota Profiling via Dynamic Breath Analysis.’

Meet Giuseppe:

Giuseppe Ferrandino holds a Bachelor's degree in Biology, graduating with the remarkable distinction of 110/110 magna cum laude. His passion for research led him to pursue a Ph.D. in Italy, where he focused on studying secondary modifier genes in congenital hypothyroidism. He received also a training at the EMBL Heidelberg with a short-term fellowship award. His outstanding academic performance during his Ph.D. earned him valuable insights into the intricate mechanisms underlying this condition.

After completing his Ph.D., Giuseppe Ferrandino embarked on a postdoctoral journey at Yale School of Medicine in the United States. During his tenure, he conducted research on membrane transport and non-alcoholic fatty liver disease in hypothyroidism. His expertise in this area resulted in the publication of five peer-reviewed articles, further expanding the scientific understanding of liver diseases.

Continuing his pursuit of scientific excellence, Giuseppe joined the Max Planck Institute in Dresden, Germany. His research at the institute focussed around two genes associated with the accumulation of fat in the liver. During this time, he gained expertise in state-of-the-art microscopy techniques, further enhancing his skill set in the field.
Currently, Giuseppe Ferrandino is utilizing his knowledge and expertise as part of the hepatology program at Owltone Medical. In his role, he provides valuable scientific support with the objective of developing non-invasive tests for liver diseases. His dedication to advancing medical knowledge and improving patient care drives his work in this area.

Microbiome Extended Session

Conclusion:

Giuseppe Ferrandino is a highly accomplished biologist with an international training in top levels academic institution. Thrived by passion Giuseppe is currently trying to make a difference at Owlstone Medical to develop novel, non-invasive, breath test.

Abstract:

Background:
Gut microbiota dysbiosis results in overproduction of metabolites that exacerbate certain chronicdiseases. For example, ethanol and 2-3-butanediol, produced by carbohydrate fermentation, were found associatedwith non-alcoholic steatohepatitis (NASH). Similarly, trimethylamine, produced from choline, is further metabolized inthe liver to trimethylamine-N-oxide, a metabolite found associated with NASH severity and that exacerbatecardiovascular diseases. We explored the feasibility of a novel approach to measure gut bacteria metabolites, whichcan be applied to assess the extent of gut bacteria metabolism in diseases populations.

Methods:
A total of 8 healthy subjects were recruited with age ≥ 18 years and body weight ≥ 50 Kg. Subjects wereinstructed to fast overnight and to not drink alcoholic beverages the day before the experiments. Breath samples werecollected before, and up to 90 minutes after ingestion of 75g of glucose with a time resolution of 5 minutes.Compounds of interest were measured using SIFT-MS with direct sampling. Results were expressed as part per billion(PPB v/v) as function of time post glucose ingestion.

Results:
Ambient measurements showed that all the investigated compounds were absent before and afterconducting the experiment. Median (M) and interquartile range (IQR) baselines levels (before glucose administration)of ethanol, propanoic acid, and acetoin (an intermediate of the 2-3-butanediol fermentation), were respectively 99.4[71.6-182.4], 13.3 [10-16.2], 3.2 [2.7, 4.2] PPB (Fig.1A). Post glucose ingestion we observed spikes of these compoundsin breath of up to respectively 8629, 153, and 20 PPB (Fig. 1B). Additional diseases associated compounds, unrelated toglucose ingestion, were also detected, such as trimethylamine (M: 42.3, IQR: 33.6-54.3 PPB) (Fig.1C), methanol (M: 443,IQR: 199-531.4 PPB) (Fig.1D), and methane (M: 26988, IQR: 10100-76213 PPB) (Fig.1E).

Conclusion:
Dynamic breath analysis
can be used for the clinical characterization of gut bacteria metabolism inhealthy and disease populations to establish correlations between metabolites and disease severity and progression,as well as interaction of gut microbiota with response to therapeutic interventions. This non-invasive method can replace the current need for blood collection allowing scaling to large cohort populations.

Alistair Taylor & Dr Kerstin Arndt-Schmitz

Boehringer Ingelheim & Owlstone Medical

‘Development of a Novel Method for Analysing Volatile Organic Compounds from Intubated Mice’

Meet Kerstin:

Kerstin's academic journey is a testament to her passion for scientific discovery. She earned her diploma in molecular biology from 2002 to 2007 and furthered her expertise with a diploma in biomedical sciences from 2004 to 2007. In 2012, she achieved a significant milestone by obtaining her Ph.D. in Biochemistry and Molecular Biology. Following her doctoral studies, Kerstin delved into the realm of research, serving as a dedicated postdoctoral scientist in biochemistry and molecular biology from 2012 to 2015. Her commitment and expertise propelled her to the role of Principal Scientist in Respiratory Research from 2018 to 2020. Kerstin's unwavering dedication to advancing scientific knowledge led her to her current position as the Associate Director of Respiratory Research since 2023, where she continues to make significant contributions to the field.

Meet Alastair, Owlstone Fellow:

Alastair joined Owlstone in 2006, leading the commercialization of chemical detectors for industrial applications. Under his guidance, Owlstone launched its first CE marked product, the Lonestar VOC Analyzer. Between 2007 and 2015, he held pivotal roles, overseeing product development in Industrial, Defence, Scientific, and Medical sectors. Alastair managed the Product Realisation Process for all commercially available products. Now an Owlstone Fellow, he provides technical expertise, ensuring design choices align with short and long-term goals and regulatory requirements. He explores emerging technologies for breath biopsy applications. Alastair holds an M’Eng from The University of Cambridge.

Abstract:

As part of their research into ILD (interstial lung disease) Boehringer Ingleheim wanted to look at breath VOC's from intubated mice. In collaboration with Owlstone a new methodology was created that adapted the Flexivent™ intubation system to collect mouse VOC's and subsequently run the samples through the standard Owlstone OMNI™ workflow. Initial data demonstrates that on-breath compounds can be identified in the mouse breath and that there is significant overlap with compounds found on human breath.

This methodology could be used in Drug Development, or applied to other intubated animal models, expediating the identification of reliable VOC biomarkers at the pre-clinical stage.

Dr Robert Rintoul

University of Cambridge
and
Royal Papworth Hospital

‘Progress in the development of a Breath Biopsy test for the early detection of lung cancer; The LuCID and Evolution studies’

Meet Robert:

Robert Rintoul is Professor of Thoracic Oncology in the Department of Oncology, University of Cambridge and Honorary Consultant Respiratory Physician, Royal Papworth Hospital. He trained in respiratory medicine in London and Edinburgh receiving his doctorate from the University of Edinburgh for work investigating mechanisms underlying resistance to chemotherapy in small cell lung cancer. He was appointed consultant in respiratory medicine specialising in thoracic oncology at Royal Papworth Hospital in 2005 before moving into the University of Cambridge in 2017. Professor Rintoul is lead clinician for cancer at Royal Papworth Hospital and Director of the Papworth Trials Unit Collaboration. He is co-lead of the CRUK Cambridge Centre Thoracic Cancer Programme and facilitates thoracic oncology research across Cambridge.

Professor Rintoul’s research is focused around clinical trials, translational research and tissue banking in malignant mesothelioma and the early detection of lung cancer. He is Chief Investigator for several clinical translational studies examining biomarkers in lung cancer. In 2014 he founded Mesobank, the UK national bioresource for malignant mesothelioma (www.mesobank.com). His work is funded by the Cambridge Biomedical Research Centre, Cancer Research UK, National Institute for Health Research and Asthma and Lung UK.
He is a member of the Roy Castle Lung Cancer Clinical Expert Group and is currently Chair of the Clinical Advisory Group of the UK Lung Cancer Coalition.

Advances in Technologies

Abstract:

Analysis of volatile metabolites in breath represents an attractive potential diagnostic modality for lung cancer. However, previous studies in this area have had various limitations that have prevented them from being translated to clinical practice. The LuCID (Lung Cancer Indicator Detection) study aimed to address these limitations by conducting the largest ever breath biomarker discovery study for lung cancer, using a state-of-the-art breath analysis approach. The results of the LuCID study suggest that individual breath biomarkers of high volatility show some association with the presence of lung cancer, particularly at advanced stages. However, the combined diagnostic performance of these biomarkers does not exceed that of clinical risk prediction models. Targeted approaches amplifying the volatile biomarker signal of metabolic pathways altered in lung cancer are likely needed to develop a breath-based screening test for lung cancer.

Kathleen Zwijsen

University of Antwerp

‘Using Ion Mobility Spectrometry for Early Mesothelioma Detection in Asbestos-Exposed Individuals: an External Validation.’

Meet Kathleen -

Kathleen Zwijsen is a PhD researcher at the University of Antwerp, specializing in biomedical sciences under the supervision of prof. Kevin Lamote and prof. Jan van Meerbeeck. Since 2022, Kathleen has been dedicated to her research on pleural mesothelioma, an aggressive asbestos-related cancer. Her work focuses on developing a non-invasive breath test for the early diagnosis of mesothelioma, aiming to improve patient outcomes through timely detection. Kathleen's research involves externally validating the breath test, which has shown promising results so far. To further advance biomarker development, she plans to utilize the test for the follow-up of professionally asbestos-exposed individuals at risk for PM. Her Respiratory Society, International Association of Breath Research and International Association for the Study of Lung Cancer.

Early Careers

Introduction: Pleural mesothelioma (PM) is a highly aggressive thoracic cancer characterized by a poor prognosis. Early diagnosis is crucial for improving patient outcomes, but due to its nonspecific symptoms, PM is often diagnosed at an advanced stage. Currently, there are no reliable diagnostic biomarkers or screening tools available for PM. However, exhaled breath analysis has emerged as a promising avenue for early detection, offering a non-invasive and easily obtainable sample that contains volatile organic compounds (VOCs). These VOCs have shown potential as biomarkers for various (patho)physiological processes [1]. To harness this potential, a breath test utilizing multicapillary column/ion mobility spectrometry (MCC/IMS) was recently developed, exhibiting an accuracy of 87% in distinguishing asbestos-exposed (AEx) individuals from PM patients [2]. Nonetheless, before implementing this test as a screening tool, it is crucial to establish its clinical utility through external validation.

Methods: Our prospective study enrolled individuals with significant occupational asbestos exposure that began at least 25 years ago. Participants consented to undergo four annual breath tests employing MCC/IMS, with the aim of adhering to the "test, re-test" principle and improving the false positivity rate. The breath tests are conducted annually over four consecutive years, as illustrated in Figure 1. Currently, we have completed the first and second sampling rounds (Figure 1). Any aberrant results from the first breath test will be confirmed after one year to be considered suspect for PM. Additionally, all participants will undergo a low-dose chest computed tomography (CT) scan correlation after the second sampling round. Treatment-naive PM patients were included as controls. Given the intended use of the breath test as an exclusive screening tool, we selected a threshold that maximizes sensitivity and negative predictive value.

Results: Our study included a total of 121 AEx individuals and 7 PM patients in the first two screening rounds. Among the AEx individuals, the breath-based model classified 55 participants as deviant in both rounds, warranting a CT scan. Notably, all 7 PM patients were correctly identified as having mesothelioma, resulting in 100% sensitivity and 100% negative predictive value for the test.

Conclusion: Our findings demonstrate that all PM patients in our study were accurately identified as having mesothelioma, while nearly half of the high-risk cohort exhibited repeated deviant results in the first two rounds of the breath-based test. These results hold the potential for initiating a screening program for early detection of PM in asbestos-exposed individuals using exhaled breath. The MCC/IMS-based breath test can serve as a valuable tool for ruling out the presence of the disease.

[1] Lamote, K., Eur Respir J, 2017
[2] Janssens, E., Cancers, 2022

Authors
K. Zwijsen (1,2), Joris Van Cleemput (3), Caroline Jonckheere (4), Tom Richart (5), K. Nackaerts (6), V. Surmont (7), J.P. van Meerbeeck (1,2,8,9) and K. Lamote (1,2)

Affiliations
(1) Laboratory of Experimental Medicine and Pediatrics, Infla-Med Center of Excellence, University of Antwerp
(2) European network of rare and low prevalent diseases (ERN-LUNG)
(3) Occupational Health Service, Eternit N.V., 1880 Kapelle-op-den-Bos
(4) Medical Service, Bekaert N.V., 8550 Zwevegem
(5) Idewe vzw, 3000 Leuven
(6) Department of Respiratory Medicine, University Hospital Gasthuisberg Leuven
(7) Department of Lung Diseases, University Hospital Ghent
(8) Department of Internal Medicine, Ghent University
(9) Department of Pulmonology & Thoracic Oncology, Antwerp University Hospital

Iris van der Sar

Erasmus University
Medical Center

‘eNose Technology in Interstial Lung Disease.’

Meet Iris -

Iris van der Sar is a medical doctor and PhD candidate at Erasmus University Medical Center in Rotterdam, the Netherlands. Her research focuses on exhaled breath analysis in Interstitial Lung Disease (ILD), with special interest in pulmonary fibrosis and sarcoidosis. Since there is no single diagnostic test for ILD and most patients experience substancial diagnostic delay, there is high need for discovery of novel biomarkers. Therefore, the primary objective of her ongoing research is to validate electronic nose technology for its application as a point-of-care diagnostic tool in clinical practice. She is also conducting exploratory research aiming to predict disease course and facilitate personalized treatment in patients with ILD using an eNose.

Early Careers

Abstract:

The group of interstitial lung disease (ILD) includes a variety of diagnoses affecting the lung interstitium. Most patients experience delay during the diagnostic process, since the ILDs are rare and patients present with generic symptoms like breathlessness and cough. Individual clinical characteristics, treatment response and disease course varies widely between and within patients of diagnosis groups. This variety requires a personalized approach for each individual. However, diagnostic and predictive biomarkers are currently lacking.
Exhaled breath might be a source of novel biomarkers. Breath analysis using an electronic nose (eNose) profiles exhaled volatile organic compounds using sensor technology. This technology has been studied in ILD over the past few years. Research showed promising results for diagnosing ILD, detecting ILD early and predicting response to ILD treatment in single and multicenter studies. International validation studies are currently ongoing. These studies might eventually lead to a clinical application to improve the diagnostic journey and personalized treatment approach for patients with ILD.

Yuta Matsuoka

Kyoto University Graduate School of Medicine

‘Breath biopsy technology targeting for ferroptosis-related volatile metabolites.’

Meet Yuta -

Yuta Matsuoka is the assistant professor from Kyoto University Graduate School of Medicine.
Throughout his career, he has advanced research concerning the development and application of lipid peroxidation analysis techniques (Anal.Chem. 2020, Nat. Commun. 2021).
Recently, he has been expanding his research into the development of breath biopsy technology focusing on "volatile oxidized lipids" and "ferroptosis".

Early Careers

Abstract:

Human exhaled air contains a large number (>1000) of volatile organic compounds (VOCs). Since individual VOCs are produced and released through specific metabolic reactions, they have attracted attention as novel non-invasive biomarkers to monitor health status and disease onset. Lipid peroxidation (LPO) has been considered one of the major origins of biological VOCs. Volatile oxidized lipids (VOLs), such as short-chain hydrocarbons and aldehydes, are released from biological lipids by oxidative fragmentation. In addition, since LPO has been recognized as an important contributor in iron-dependent cell death, “ferroptosis” and the pathogenesis of ferroptosis-related diseases, the analytical technologies for VOLs will enable futuristic medical diagnosis to predict disease risk from “exhaled breath”. However, the detailed generation mechanisms of VOLs have not been well understood. Therefore, the profiles of biological VOLs generated in ferroptosis have been unclear. To address this challenge, we here constructed the structural library of VOLs and also identified ferroptosis-specific volatile metabolites. Furthermore, we applied our developed library to the comprehensive analysis of biological VOLs released from nonalcoholic steatohepatitis (NASH)-model mice, and found that the levels of several ferroptosis-specific VOLs, especially ω-6 PUFA derived VOLs, in mouse biogas were elevated with NASH progression. Our developed methodologies are expected to be innovative tools for non-invasive detection of ferroptosis-related diseases from exhaled breath.

Authors: Matsuoka Y.(1) and Sugiura Y.(1)

Affiliations: Graduate School of Medicine, Kyoto University, Japan

Ask the Expert

Panel Discussion

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 for debate and discussion around the answers with other experts.

In 2023, our Ask the Expert session will center on achieving better standardization in breath research. Chaired by our CEO and Co-Founder Billy Boyle, this discussion will explore innovative strategies and analytical approaches to enhance standardization within the field.

Billy Boyle

CEO and Co-Founder
Owlstone Medical

Ethan McBride

Los Alamos National Laboratory

Brooke Kaiser

Pacific Northwest National Laboratory

Mangilal Agarwal

Indiana University-Purdue University (IUPUI)

Michael Wilde

University of Plymouth