13th International Conference

ABR
BREATH SUMMIT 2022

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BREATH SUMMIT 2022
12|15 JUNE 2022
PISA - ITALY

Breath Summit 2022

12|15 June 2022

Pisa - Italy

Conference Programme and Book of Abstracts

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Conference website: iabr.dcci.unipi.it

Contents

Conference committees

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- Prof. Fabio Di Francesco Chair - University of Pisa
- Prof. Cristina Davis, University of California (US)
- Dr. Wolfram Miekisch, University Medicine Rostock (DE)
- Prof. Jane Hill, The University of British Columbia (CA)
- Dr. Nandor Marczin, Imperial College London (UK)
- Prof. Marieann Högman, Uppsala University (SE)
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- Dr. Dorota Ruszkiewicz, Loughborough University (UK)
- Prof. Dr. Alexander Mœller, Kinderspital Zürich (CH)
- Dr. Raed Dweik, Cleveland Clinic (US)
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- Dr. Tobias Bruderer - University of Pisa
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- Dr. Bernardo Melai - University of Pisa
- Alessio Lenzi - University of Pisa

Conference sessions

Session 1 **From Crete to Pisa – two decades of breath research**

Chair: Marieann Högman, Nandor Marçzin

Session 2 **Breath biomarkers of Sars Cov-2 infection**

Chair: Renelle Myers, Makoto Sawano

Session 3 **Spectroscopic methods for breath analysis**

Chair: Andreas Güntner, Ines Weber

Session 4 **Sensors and sensor systems**

Chair: Rosamaria Capuano, Nick Rothbart

Session 5 **Standardization of breath collection and instrumentation**

Chair: Paul Thomas, Sean Harshman

Session 6 **Breath biomarkers of microbial infections**

Chair: Jane Hill, Patricia Fuchs

Session 7 **Breath tests targeting unmet clinical needs**

Chair: Jochen Schubert, Inger Lise Gade

Session 8 **Data analysis and interpretation**

Chair: Agnieszka Smolinska, Robert van Vorstenbosch

Session 9 **Analytical methods for breath biomarker detection**

Chair: Veronika Ruzsanyi, Bogusław Buszewski

Session 10 **Breath analysis and cancer research**

Chair: George Hanna, Daria Slefarska-Wolak

Session 11 **Five shades of breath analysis**

Chair: Wolfram Miekisch, Jonathan Beauchamp

Session 12 **Volatile signatures of asthma in breath**

Chair: Alexander Mœller, Renate Kos

Session 13 **Young scientist session I**

Chair: Dorota Ruszkiewicz, Tommaso Lomonaco

Session 14 **Young scientist session II**

Chair: Franziska Lochmann, Veronika Ruzsanyi

Session 15 **Skin volatilome and other stories**

Chair: Aoife Morrin, Tobias Bruderer

List of titles

Plenary lectures

Invited talks

Oral presentations

Flash poster presentations

Marek Jackowski	–	Protocols for colorectal cancer biomarkers
Mahya Khaki & Matthias G. Friedrich	–	The predictive value of the heart rate response to breathing maneuvers for significant coronary artery disease
Agapios Agapiou	–	VOCs and PM in confined environments
Tanja Zivkovic Semren	–	Workflow Development for Real-Time Exhaled Breath Analysis by Secondary Electrospray Ionization coupled to High Resolution Mass Spectrometry
Tara Lovestead	–	A pilot study to determine if THC can be detected in breath aerosols collected from legal market cannabis users with an impaction filter device
Marilena Giglio	–	Quartz-enhanced photoacoustic detection of ammonia in exhaled breath
Giuseppe Ferrandino	–	Pre-clinical exogenous volatile organic compounds (EVOCs) Probes screening and optimization for chronic liver diseases detection
Sean W. Harshman	–	Investigation of an individual with low exhaled isoprene
Marieann Högman	–	Alveolar nitric oxide in COPD – a 2-year follow-up
Leo Rührmund	–	Data visualization for real time mass spec-based breath analysis in clinical setups
Cedric Wüthrich	–	Online SESI-HRMS breath analysis after a nutritional intervention challenge
David M. Fothergill	–	Exhaled breath condensate profiles of US Navy divers following prolonged hyperbaric oxygen (HBO) and nitrogen-oxygen (Nitrox) chamber exposures
Ning Sun	–	A core breath profile of healthy non-human primates
Simonetta Capone	–	Analysis of urinary volatile organic compounds by electronic nose and GC/MS for prostate cancer diagnosis
Austin Meister	–	Detection of SARS-CoV-2 Omicron infection in exhaled breath from out-patients with mild respiratory symptoms
Francesco Segrado	–	Mass spectrometry profiling of exhaled breath of smokers to identify a signature related to tobacco use
David J. Mager	–	Towards targeted exhaled breath analysis for young children in CF care to detect bacteria in the lungs
Dominic Sandhu	–	Utilising computational methods to determine an idealised lung clearance index
Joris Meurs	–	Development and validation of a proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) method for analysis of short-chain fatty acids (SCFAs) in exhaled breath

Raj Attariwala	–	Correlation of breath and blood cannabis levels using custom-made breath sample collection and analysis method
Evangelia Sakkoula	–	Monitoring dietary status and cognitive functioning in children through exhaled breath analysis
Pritam Sukul	–	Recommended methods for safe breath analysis under highly infectious respiratory conditions

Poster session

Marek Jackowski	–	Protocols for colorectal cancer biomarkers
Tomasz Ligor	–	Extraction and identification of volatile organic compounds in fecal samples by SPME and dynamic headspace thermal extraction followed by GCMS
Mahya Khaki & Matthias G. Friedrich	–	The predictive value of the heart rate response to breathing maneuvers for significant coronary artery disease
Agapios Agapiou	–	VOCs and PM in confined environments
Tanja Zivkovic Semren	–	Workflow Development for Real-Time Exhaled Breath Analysis by Secondary Electrospray Ionization coupled to High Resolution Mass Spectrometry
Rosaria Orlandi	–	Secondary Electrospray Ionization– High Resolution Mass Spectrometry (SESI-HRMS) profiling of exhaled breath of head and neck cancer patients for clinical practice
Tara Lovestead	–	A pilot study to determine if THC can be detected in breath aerosols collected from legal market cannabis users with an impaction filter device
Marilena Giglio	–	Quartz-enhanced photoacoustic detection of ammonia in exhaled breath
Ran Wang	–	Exhaled volatile organic compounds during inhaled mannitol challenges in adults
Giuseppe Ferrandino	–	Pre-clinical exogenous volatile organic compounds (EVOCs) Probes screening and optimization for chronic liver diseases detection
Elodie Lamy	–	Annotation of biomarkers in exhaled breath: combining real-time mass spectrometry and two-dimensional chromatography-mass spectrometry
Sean W. Harshman	–	Investigation of an individual with low exhaled isoprene
Marieann Högman	–	Alveolar nitric oxide in COPD – a 2-year follow-up
Andreas T. Güntner	–	Selective monitoring of breath isoprene by a sensor during exercise and at rest
Andreas T. Güntner	–	Monitoring rapid metabolic changes in health and type-1 diabetes with breath acetone sensors

Leo Rührmund	–	Data visualization for real time mass spec-based breath analysis in clinical setups
Cedric Wüthrich	–	Online SESI-HRMS breath analysis after a nutritional intervention challenge
Stamatios Giannoukos	–	Development and testing of a vapor generator for quantification of exhaled breath metabolites
David M. Fothergill	–	Exhaled breath condensate profiles of US Navy divers following prolonged hyperbaric oxygen (HBO) and nitrogen-oxygen (Nitrox) chamber exposures
Ronja Weber	–	Effects of a volatile compound filter on breath profiles measured by online high-resolution mass spectrometry
Mitchell M. McCartney	–	Diagnosis of SARS-CoV-2 infection from exhaled breath volatiles using GC-MS
Hamad A. Alzoman	–	The effect of sleeve gastrectomy on halitosis
Ning Sun	–	A core breath profile of healthy non-human primates
Simonetta Capone	–	Analysis of urinary volatile organic compounds by electronic nose and GC/MS for prostate cancer diagnosis
Breanna Dixon	–	Metabolic phenotyping of acquired ampicillin resistance using microbial volatiles from *Escherichia coli* cultures
Chad Schaber	–	Breath Biopsy OMNI: Advanced Global Breath VOC Analysis
Austin Meister	–	Detection of SARS-CoV-2 Omicron infection in exhaled breath from out-patients with mild respiratory symptoms
Francesco Segrado	–	Mass spectrometry profiling of exhaled breath of smokers to identify a signature related to tobacco use
Sam Bonsall	–	Headspace analysis of asbestos exposed mesothelioma cell lines
David J. Mager	–	Towards targeted exhaled breath analysis for young children in CF care to detect bacteria in the lungs
Dominic Sandhu	–	Utilising computational methods to determine an idealised lung clearance index
Sara Barreto	–	The volatilome of human pluripotent stem cells using selected ion flow tube-mass spectrometry
Esenkova Ekaterina	–	Correlation of breath metabolites and microbiome in IBS population
Joris Meurs	–	Development and validation of a proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) method for analysis of short-chain fatty acids (SCFAs) in exhaled breath
Raj Attariwala	–	Correlation of breath and blood cannabis levels using custom-made breath sample collection and analysis method
Evangelia Sakkoula	–	Monitoring dietary status and cognitive functioning in children through exhaled breath analysis
Stig Lytke Brejl	–	A Feasibility study using the ETL CoronaCheck® to identify incident cases of SARS-CoV-2: Find SARS-CoV-2

Ran Wang	–	Exhaled volatile organic compounds in suspected asthma - preliminary data from the RADicA study
Waqar Ahmed	–	Generating pooled quality control samples for VOC analysis by TD-GC-MS
Waqar Ahmed	–	Direct thin-film microextraction of volatile metabolites from an air-liquid interface culture infected with *Staphylococcus aureus*
Waqar Ahmed	–	Volatile metabolite profiling of morphological changes in *Candida albicans*
Pritam Sukul	–	Recommended methods for safe breath analysis under highly infectious respiratory conditions

General programme

(All times are Central European Summer Time)

Sunday	June 12, 2022
	Room 27
8h30 8h45	Registration
8h45	Short course
11h10	Coffee break
11h30	Short course
13h00	Lunch break
14h00 15h45	Short course
	Auditorium
14h30	***Registration***
16h00	Opening ceremony (Fabio Di Francesco, Cristina Davis)
	Session 1: From Crete to Pisa – Two decades of breath research Chair: Marieann Högman and Nandor Marçzin
16h10	Invited talk: Nandor Marçzin "Role of the Crete NATO Advanced Study Institute in collaborative breath analysis"
16h30	Invited talk: Marieann Högman "From Crete to Pisa – what has happened in NO research"
16h50	Invited talk: Wolfram Miekisch "Two decades of breath VOC research focused on medical applications – clinical studies, technical developments, basics of VOC exhalation and data interpretation – Lessons learned from Crete 2000 to Pisa 2022 –"
17h20	Invited plenary: Anil S. Modak "Why have only a handful of breath tests made the transition from R&D to clinical practice?"
17h50	Invited pleanary: George Hanna "Breath analysis at the frontiers of cancer research"
18h30	
19h45 22h00	Welcome cocktail

Monday	June 13, 2022	
8h30	***Registration***	
9h00	Invited plenary : Jane E. Hill "New frontiers: Introducing the human breath atlas"	
	Auditorium	**Room 27**
	Session 2: Breath biomarkers of Sars Cov-2 infection Chair: Renelle Myers, Makoto Sawano	**Session 3: Spectroscopic methods for breath analysis** Chair: Andreas Güntner, Ines Weber
9h30	Makoto Sawano	Nick Rothbarth
9h50	Rasmus Remy	Mike Mirov
10h10	Madiha Malik	Miloš Selaković
10h30	Veronika Pospisilova	Gunnar Johanson
10h50	**Coffee break**	
	Session 2: Breath biomarkers of Sars Cov-2 infection Chair: Renelle Myers, Makoto Sawano	**Session 4: Sensors and sensor systems** Chair: Rosamaria Capuano, Nick Rothbart
11h20	Renelle Myers	Ines Weber
11h40	Amalia Berna	Andreas Güntner
12h00	Nicholas Kenyon	Alexander Pospelov
12h20	Laura Miles	Gennadii Kamarchuk
12h40		Rosamaria Capuano
13h00	**Lunch break**	
	Session 5: Standardization of breath collection and instrumentation Chair: Paul Thomas, Sean Harshman	**Session 6: Breath biomarkers of microbial infections** Chair: Jane Hill, Patricia Fuchs
14h30	Jens Herbig	Agnieszka Smolinska
14h50	Sean Harshmann	Antao Gao
15h10	Y. Lan Pham	Patricia Fuchs
15h30	Karl Unterkofler	Nele Kemnitz
15h50	Kavita Jeerage	Waqar Ahmed
16h10	Chad Schaber	
16h30	Coffee break	
17h00	Flash poster presentations (Chair: Dr. Pietro Salvo, Dr. Denise Biagini)	
17h30	Poster session	
20h00	Dinner International Committee	

Tuesday	June 14, 2022	
8h30	***Registration***	
9h00	Invited plenary : Cristina Davis "Volatiles in biological systems shift with disease state: a universal paradigm connecting human and animal breath analysis to plant and agriculture diagnostics"	
	Auditorium	**Room 27**
	Session 7: Breath tests targeting unmet clinical needs Chair: Jochen Schubert, Inger Lise Gade	**Session 8: Data analysis and interpretation** Chair: Agnieszka Smolinska, Robert van Vorstenbosch
9h30	Tobias Walser	Camille Roquencourt
9h50	Fabio Di Francesco	Robert van Vorstenbosch
10h10	Renate Kos	Celia Mallafré-Muro
10h30	Nicholas Smith	Monika Śmiełowska
10h50	**Coffee break**	
	Session 9: Analytical methods for breath biomarker detection Chair: Veronika Ruzsanyi, Bogusław Buszewski	**Session 10: Breath analysis and cancer research** Chair: George Hanna, Daria Slefarska-Wolak
11h20	Bogusław Buszewski	Jolanda Palmisani
11h40	Wolfgang Vautz	Sarah Haywood-Small
12h00	Thomas Wortelman	Eline Schillebeeckx
12h20	Antonello Laricchiuta	Kiran Sankar Maiti
12h40		Theo Issitt
13h00	**Lunch break**	
	Session 11: Five shades of breath analysis Chair: Wolfram Miekisch, Jonathan Beauchamp	**Session 12: Volatile signatures of asthma in breath** Chair: Alexander Mœller, Renate Kos
14h30	Inger Lise Gade	Mahmoud Abdel-Aziz
14h50	Pritam Sukul	Ronja Weber
15h10	Sarah Dowling	Yoni E. van Dijk
15h30	Simonetta Capone	Alexander Möller
15h50	Joris Meurs	Andrei Malinovschi
16h10	Coffee break	
16h40	Focus groups	
17h45	Amann award announcement	
18h00 18h30	Awardee plenary session	
20h00	Transfer to Villa Scorzi	
21h00	Social dinner	
0h00	Back from Villa Scorzi	

Wednesday	June 15, 2022	
9h00	***Registration***	
9h30	Invited plenary : Paul Thomas "Time's arrow, and the moving target of your phenome. A brief meditation on root causes of variability and irreproducible observations in breath research"	
	Auditorium	**Room 27**
	Session 13: Young scientist session I Chair: Dorota Ruszkiewicz, Tommaso Lomonaco	**Session 14: Young scientist session** II Chair: Franziska Lochmann, Veronika Ruzsanyi
10h10	Hannah Schanzmann	Daria Slefarska-Wolak
10h30	Lorenzo Petralia	Eline Janssens
10h50	**Coffee break**	
	Session 13: Young scientist session Chair: Dorota Ruszkiewicz, Tommaso Lomonaco	**Session 15: Skin volatilome and other stories** Chair: Aoife Morrin, Tobias Bruderer
11h20	Ning Sun	Kathleen Zwijsen
11h40	Iris van der Sar	Federico Vivaldi
12h00	Nynke Wijbenga	Aoife Morrin
12h20	Carmen Bax	Tobias Bruderer
12h40	Franziska Lochmann	Amy Worrall
13h00	**Lunch break**	
14h30	IABR president election	
15h10	Best poster awards	
15h30 16h30	Round table and greetings	

Social programme

Welcome Cocktail

Date	Sunday 12
Time	Evening (from 19h45)
Place	DOMUS COMELIANA
Location website	**www.domuscomeliana.com**

Meet old and new colleagues for an informal welcome reception, held at the DOMUS COMELIANA location close to the famous tower of Pisa in Piazza dei Miracoli. The reception is included in the registration fee.

Social Dinner

Date	Tuesday 14
Time	Evening (20:00 – midnight)
Place	VILLA SCORZI
Location website	**www.villascorzi.com/en**

Social Dinner will take place in the fourteenth century VILLA SCORZI location which is located in the shadow of the famous Certosa of Calci, surrounded by the enchanting setting of the mountains and the countryside of Pisa.

All photos © *www.villascorzi.com*

Social Trip to Florence

Date	Thursday 16
Time	Whole day
Place	FIRENZE

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A walking tour of FLORENCE is planned on June 16th that will allow you to discover the charm of one of the most beautiful cities in Italy and experience the heart and soul of the Italian Renaissance! The breath-taking beauty of the Duomo and its massive dome will unveil before you reach Signoria Square - a real 'open air' museum- and Ponte Vecchio - one of the oldest bridges in Europe. The tour will then delve into the charming Medieval district, to admire ancient tower-houses hidden among narrow streets and alleys, and lead you to the Oltrarno, on the other side of the river, for a taste of one of the most famous Italian style gardens in Europe, the Boboli Gardens, built by the Medici family in the XVIth century and boasting beautiful Renaissance fountains, statues, and artificial grottoes! After a free shopping time for souvenirs seekers, we will go back to Pisa to enjoy the charming Luminara of San Ranieri.

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Luminara (Pisa by candlelight)

Date	Thursday 16
Time	Evening
Place	PISA

Festivities to celebrate San Ranieri in Pisa include the beautiful “LUMINARA” on the evening of June 16th.

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Plenary Lectures

Ponte di Mezzo, Lungarni Pisani

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Anil S. Modak

Cambridge Isotope Laboratories Inc., 50 Frontage Road, Andover, MA 01810, USA

Why have only a handful of breath tests made the transition from R&D to clinical practice?

Sunday 12, 17h20 – 17h50, Auditorium

Over the last five decades, exogenous and endogenous volatile organic compounds found in human breath have been used in several clinical studies to non invasively evaluate their potential in diagnosing/detecting or monitoring human diseases [1]. Only a handful of breath tests using endogenous and exogenous biomarkers have been approved by regulatory boards and made the transition from research to routine use in clinics and hospitals.

Despite the potential of these breath tests being non invasive, easy to administer with rapid measurement and analysis of breath components, the lack of clinical utility due to low specificity has prevented the development and approval by regulatory boards to make the transition from research to routine use in clinics and hospitals. Some promising breath tests using 13C probes for unmet clinical have also fallen by the wayside due to the reluctance of pharmaceutical/biotech companies to invest in their development.

^{13}C-probes as well as the eVOC probes can be used for the evaluation of various drug metabolizing CYP450 enzyme activity which will aid physicians in personalizing medications instead of the current trial and error paradigm in clinical practice. With these labeled or unlabeled probes, the metabolic pathway and the enzymes involved in the origins of the breath biomarker (13CO2 or metabolites) are well known and unambiguous.

Despite extensive research and thousands of published papers in the field of exogenous and endogenous breath volatiles for diagnosing diseases over 5 decades only five have been FDA/EMA approved and used in routine clinical practice. Several hurdles need to be overcome to get breath tests from research phase into the clinic.

Researchers cannot keep using different methods of breath sample collection, different methods for analysis of samples, not conclusively identifying the breath biomarkers and expect physicians and nurses to use them routinely in clinical practice or regulatory boards (FDA, EMA etc) to approve them.

References

[1] Breathborne Biomarkers and the Human Volatilome, Second Edition, Eds. Jonathan Beauchamp Cristina Davis, Joachim Pleil, Elsevier BV 2020. https://doi.org/10.1016/B978-0-12-819967-1.00017-7.

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George Hanna

Breath analysis at the frontiers of cancer research

Sunday 12, 17h50 – 18h30, Auditorium

Jane E. Hill

Department of Chemical and Biological Engineering, The University of British Columbia, Canada

New Frontiers: Introducing The Human Breath Atlas

Monday 13, 9h00 – 9h30, Auditorium

In recent decades, the field of breath analysis has demonstrated proof-of-concept for non-invasive diagnostics and monitoring for a variety of diseases, disorders and exposures. The importance of our work has been tragically underscored by the SARS-CoV-2 pandemic, and the encouraging emergence of rapid breath-based diagnostics. We have used targeted and discovery paradigms to identify breath biomarkers of significance, and regulatory agencies are now providing pathways for breath analysis to join traditional diagnostic measures. Concurrently, gold standard analytical equipment to survey breath biomarkers has significantly advanced.

Our field of breath analysis is currently at an inflection point. Many studies have been limited in scope due to funding, timing, resources and other requirements. We frequently observe studies of around 50-100 subjects, but never more than hundreds of subjects. These are valiant efforts and clearly demonstrate proof-of-concept for our field. But clinical studies of this scale are insufficient to motivate the large-scale investment required to take our entire field to the next level.

We see an urgent need for a worldwide endeavor to define a large-scale baseline of human breath metabolomics. We envision a global network of research groups utilizing standardized sampling and analysis methods with quality assurance and control, yielding a publicly available human breath database; we name this repository The Human Breath Atlas. We imagine a variety of stakeholders and funding agencies, spanning NGOs, national governments, and private industry. We also envision core facilities to operationalize and industrialize sample collection and analysis, while promoting common data standards and building collaborations with academic labs, supported by high-quality software and technical infrastructure. This paradigm shift towards a community and scaled approach to large-scale breath biomarker discovery will unleash our full capacity as a field, analogous to the human genome effort. With retrospective and prospective data analysis enabled by cutting-edge engineering and machine learning, there is much to learn from a broad and comprehensive study. We will briefly describe our vision for the scale and scope of such a community effort.

Jane E. Hill (1), Heather Bean (2), Jonathan Beauchamp (3), Raed Dweik (4), Jens Herbig (5), Cristina E. Davis (6), Makoto Sawano (7), C.L. Paul Thomas (8) and Alex Wiltschko (9)

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(7) Department of Emergency Medicine and Critical Care, Saitama Medical Center Hospital, Saitama Medical University, Japan
(8) Emeritus Professor, Centre for Analytical Science, Chemistry, Loughborough University, UK
(9) Google Research, Brain Team, Cambridge, MA, USA

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Cristina Davis

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Volatiles in biological systems shift with disease state: a universal paradigm connecting human and animal breath analysis to plant and agriculture diagnostics

Tuesday 14, 9h00 – 9h30, Auditorium

Volatile organic compounds (VOCs) are generated by almost all biological systems, and are now thought to represent end products associated with organism physiology and cellular metabolism. This applies to many different types of systems across humans, animals, bacteria, fungus, trees and plants. In humans, many hundreds of chemical compounds have been detected in exhaled breath, skin, urine and feces and many of these appear to relate to health and disease status. Because of the potential to exploit this signal, breath analysis provides a wide range of opportunities for diagnosis of pathophysiological conditions in a non-invasive and potentially inexpensive way.
Likewise, plants are known to off-gas a plethora of VOCs, some of which may also be associated with health status and are deeply involved in plant/insect signaling. Evidence is mounting that specific odor profile shifts are associated with bacterial or viral infections – and this mirrors the discovers that we have in the human breath analysis community. By monitoring these emitted chemicals, we can develop tools for early stage asymptomatic diagnostics that can lead to early therapy and treatment in agriculture systems as well.

This talk explores the strong parallels between human breath VOCs and those same types of biomarkers in the agriculture application space. We have much to learn from both of these communities.

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Paul Thomas

Analytical Science, Centre of Analytical Science, Department of Chemistry, Loughborough University, United Kingdom

Time's arrow, and the moving target of your phenome. A brief meditation on root causes of variability and irreproducible observations in breath research

Wednesday 15, 9h30 – 10h10, Auditorium

A synoptic review of COVID-19 detection research, development of acute CBRN breath tests, and the peppermint initiative studies, leads to the suggestion that a conceptual framework based on phenoconversion and phenoreversion will be useful in the delivery of higher impact phenomic breath research. The aim of this paper is to describe these concepts and consider their potential effects within breath testing.

Root cause analysis of poor reproducibility in breath research studies raises questions such as:

- What if poor reproducibility in breath research is due to underlying mechanisms that cannot be addressed by satisfactory quality assurance and quality control alone?
- What if our underlying assumptions on the nature of change, and effects of interventions, in breath biochemistry are incomplete, or not valid?
- And, what if a combination of ever more analytically-sensitive platforms with our data pipelines has the potential to edit out previous findings unintentionally?

The dynamic and multi-factor nature of phenoconversion is considered, and in particular, constructive, and destructive chemical interference processes are postulated as potential sources of variability and irreproducibility. It would appear that incompletely defined, and uncontrolled, phenoconversion and phenoreversion processes within a study cohort have the potential to obscure and confound study protocols, and combined with multiple individualised factors (e.g. environment, behavioural, treatments, diets) they represent a potent source of variability.

Models of randomised stratified sampling are considered, and the utility of time-series study designs and complementary biofluid samples, as a way of better alignment of phenoconversion processes across cohorts are proposed. Importantly, enabling such approaches requires standardised data reporting and processing, rigorously described and executed QA/QC protocols, and completely defined Analytical Quality by Design (AQbD) work-flows and pipelines.

C. L. Paul Thomas & Dorota M. Ruszkiewicz (1)

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Invited talks

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Nandor Marçzin

Role of the Crete NATO Advanced Study Institute in collaborative breath analysis

Sunday 12, 16h10 – 16h30, Auditorium

Marieann Högman

Department of Medical Sciences, Uppsala University, Uppsala, Sweden

From Crete to Pisa – what has happened in NO research

Sunday 12, 17h20 – 17h50, Auditorium

Background: Twenty years ago, nitric oxide (NO) had already been discovered in exhaled gas [1] and likewise its flow dependency [2]. It was known that the human tissue produces nitric oxide with the help of enzymes. One of them, inducible NO synthase, is upregulated during inflammation to produce high amounts of NO in the tissue. This led the scientists to look for exhaled NO (F_ENO) in persons with inflammatory disease states, i.e. asthma [3].

To be able to compare F_ENO levels between research groups it was necessary to standardize the measurements. The European Respiratory Society and the American Thoracic Society had published their recommendations. Finally in 2005, the joint paper "Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide" was published [4]. Today, this is still the main document referred to for the NO measurements. However, the F_ENO measurement will not reveal from where in the lung the NO is produced. At the Crete meeting, the mathematics for NO lung dynamics were presented together with data from healthy persons, smokers, and patients with asthma and COPD [5]. It took until 2017 before the standardisation for the flow and quality control standards for the linear and the non-linear models of NO lung dynamics to be published [6].

The technical development has gone from environmental NOx analysers to rapid and accurate chemiluminescence analysers with built in flow measurements. Other technologies have also been presented, i.e. electrochemical cells and laser. We now have handheld devices for the doctors' offices and clinical practice guidelines for the interpretation of F_ENO [6]. The use of F_ENO measurements has also been introduced into clinical treatment guidelines for asthma. Hopefully, we will shortly have reference equations for healthy persons.

Conclusion: It took a long time to get the F_ENO measurement into the clinical setting. What have we learnt and what could we have done differently?

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Wolfram Miekisch

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Two decades of breath VOC research focused on medical applications – clinical studies, technical developments, basics of VOC exhalation and data interpretation – Lessons learned from Crete 2000 to Pisa 2022 –

Sunday 12, 16h50 – 17h10, Auditorium

Starting in 1996 with first clinical studies in mechanically ventilated critically ill patients, our research was focused on exhaled VOC profiles in patients suffering from acute lung failure (ARDS /ALI). At the NATO meeting in Crete VOC analysis was still considered as a sideshow in relation to NO and breath condensate research, but we got into contact with important and inspiring researchers and ideas from the whole field. In the following years, we conceived many VOC related clinical studies in spontaneously breathing and mechanically ventilated patients. RoMBAT (Rostock Medical Breath Analysis and Technologies) improved sampling and analytical methods for breath profiling at the bedside and applied these techniques to different cohorts of patients (ARDS, SIRS, sepsis, cancer, heart surgery, brain injury…). In addition, breath analysis was extended to large animal models and volatiles emitted from (cell or bacterial) cultures to get better understanding of origins and exhalation kinetics of breath VOC biomarkers. This knowledge was used to identify and solve questions related to blood- breath correlations, confounding variables and data over fitting. The application of breath resolved real time monitoring further enhanced our knowledge on regular physiological effects on exhaled VOC concentrations and enabled monitoring of dynamic exhaled profiles in (ICU) patients during treatment/ therapy.
This lecture will give an overview on more than 20 years VOC research in a clinical context, research gaps identified, problems solved and lessons learned.

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Oral presentations

"Fontana dei Putti" (statue by Giuseppe Vaccà), Piazza dei Micoli, Pisa

© Ph. Francesca G. Bellagambi

Overview on Piazza dei Micoli, Pisa

© Ph. Carlo Cafferini

Monday 13 June

Session 2 Breath biomarkers of Sars Cov-2 infection

Chair: Renelle Myers, Makoto Sawano

Session 3 Spectroscopic methods for breath analysis

Chair: Andreas Güntner, Ines Weber

Session 4 Sensors and sensor systems

Chair: Rosamaria Capuano, Nick Rothbart

Session 5 Spectroscopic methods for breath analysis

Chair: Paul Thomas, Sean Harshman

Session 6 Spectroscopic methods for breath analysis

Chair: Jane Hill, Patricia Fuchs

Makoto Sawano Monday 13, Auditorium, 9h30

Center for Advanced Emergency Medicine and Critical Care, Saitama Medical Center, Saitama, Japan

RT-PCR Diagnosis of COVID-19 from Exhaled Breath Condensate: wild-type and Delta variant

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(2) Department of Clinical Laboratory Medicine, Saitama Medical Center, Saitama, Japan
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Background: Current diagnostic testing for coronavirus disease 2019 (COVID-19) is based on detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swab samples by reverse transcription polymerase chain reaction (RT-PCR). However, the test is associated with increased risks of viral dissemination and environmental contamination and shows relatively low sensitivity. Given that COVID-19 is transmitted via exhaled aerosols and droplets, and that exhaled breath condensate (EBC) is an established modality for sampling exhaled aerosols, detection of SARS-CoV-2 in EBC offers a promising diagnostic approach. The objective of the study was to quantify the viral load in EBC collected from COVID-19 patients and to validate the feasibility of SARS-CoV-2 detection from EBC as a diagnostic test for the infection.
Method: EBC samples were collected using Rtube® from 85 spontaneously breathing COVID-19 patients (45 wild type infected and 40 Delta variant infected) after admission to Saitama Medical Centre Hospital. Viral RNA loads were quantified by RT-PCR targeting the E gene. Changes in detection rates and viral RNA loads relative to patient characteristics (days since symptom onset, strain, etc.) were statistically evaluated employing multi-variate linear and logistic regression models to adjust confounding factors.
Results: Infection by Delta variant and short period since symptom onset were significant independent predictors of high viral RNA load and detection rate in EBC. Conclusion: SARS-CoV-2 RNA load in EBC relative to days since onset was significantly higher in patients infected with Delta variant compared to wild type. The study supported the feasibility of viral RNA detection in EBC as a diagnostic test for COVID-19 within 2 days (sensitivity ≥86%) or 8 days (sensitivity ≥75%) after symptom onset for patients infected with wild type or Delta variant, respectively.

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Nick Rothbart Monday 13, Room 27, 9h30

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Human breath analysis by millimeter-wave gas spectroscopy in comparison to GC-MS

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(6) IHP—Leibniz-Institut für Innovative Mikroelektronik, Frankfurt (Oder), Germany

Background: For a wide use of exhaled human breath analysis, compact, easy-to-use low-cost systems that can detect a large variety of molecules with high sensitivity and high specificity are required. Millimeter-wave gas spectroscopy (MMWGS) is a new method for this application that combines all of these characteristics. Thus, it has the potential to be used for medical screenings as well as to contribute complementary knowledge to breath research. The proof of principle for MMWGS for breath analysis has already been provided [1, 2]. In this work, we demonstrate the analysis of a larger number of samples from a medical environment and validate the results by comparison to the well-established method gas chromatography-mass spectrometry (GC-MS).

Methods: We have analyzed 28 sets of samples from COPD patients. Each set consists of four identical samples, of which two were analyzed by a reference GC-MS system and two by our MMWGS system. It is based on a compact gas absorption cell, a receiver and a transmitter emitting radiation at a frequency around 250 GHz [3]. The sample contents were released in the gas cell where they were probed at distinct frequencies according to molecular fingerprints. We have analyzed and compared the abundances of acetonitrile, acetaldehyde, ethanol, and acetone.

Results: The results of both methods agree very well for acetonitrile, ethanol and acetone with correlation coefficients of R=0.58, R=0.93 and R=0.83, respectively. In the case of acetaldehyde, we didn't observe a correlation between both methods, which is likely affected by the very low abundances and the sampling process. The reliability of both methods in our study was very similar for both methods, as investigated by the variations within the duplicate samples.

Conclusions: MMWGS is a promising method for the analysis of exhaled human breath because it can identify polar molecules in a gas mixture with high sensitivity and high specificity. We have successfully validated the results with an established method which is a very important step towards the application in breath research or in doctor's offices. An additional advantage of MMWGS is the simple operation principle which allows for compact systems that can contribute to the advancing progress of breath research.

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Rasmus Remy Monday 13, Auditorium, 9h50

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Profiling of exhaled volatile organics in the screening scenario of a COVID-19 test centre

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Background: Due to their immediate exhalation after generation at the cellular level exhaled volatile organics (VOCs) may provide real-time information on pathophysiological mechanisms and host response to infections.

Methods: We conducted PoC real-time mass-spectrometry based consecutive breath profiling in 708 subjects under conditions of a realistic screening scenario in a COVID-19 test center. Recruited subjects were grouped for further comparisons, based on PCR confirmed infection status (infected by SARS-CoV-2 or other respiratory pathogens and healthy), RTqPCR cycle threshold (Ct) values and presence or absence of flu like symptoms.

Results: Exhaled VOC profiles of SARS-CoV-2 positive cases (n=36) differed from healthy (n=256) and other respiratory infections (n=416). VOC concentrations also differed between symptomatic and asymptomatic subjects. Unlike previous studies, we observed suppressions of most breath markers in COVID-19. Under-expressions of butyric acid was found as SARS-CoV-2 infection characteristic. Irrespective of tracible disease symptoms, dimethyl sulfide decreased with increasing viral loads. Other VOCs linked to immune host response were over-expressed in cases with respiratory pathogens other than SARS-CoV-2.

Conclusions: Alike recent metagenomic and bio-chemical reports, breath profiles of exhaled VOCs mirror interactions of virus with hosts' cellular metabolism, immune homeostasis of the systemic microbiome. Decreased exhalations of specific volatiles can be attributed to suppressive effects of SARS-CoV-2 onto gut- or pulmonary microbial metabolism. Thus, breath analysis holds potential for monitoring SARS-CoV-2 infections rather than for primary diagnosis. Breath VOC profiling offers knowledge on host-virus crosstalk beyond conventional understanding of microbiology and non-invasive monitoring of pathobiological events linked to viral entry and disease manifestation.

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Mike Mirov Monday 13, Room 27, 9h50

IPG Photonics – Southeast Technology Center, Birmingham, AL 35211, USA

Cr:ZnS laser-based dual comb spectroscopy: A novel platform for breath analysis

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Abstract: Laser Dual Comb Spectroscopy (DCS) is a high resolution, high sensitivity, and massively parrallel spectroscopic technique that can enable real time, point of care, parallel detection and quantification of multiple biomarkers in breath without sample pre-concentration. To date, dual-comb spectroscopy laser platforms were bulky, expensive, and bandwidth limited. Recently, Cr:ZnS laser frequency combs technology has made great strides in addressing these challenges.

Background: Traditional broadband DCS systems are based on down-conversion of readily available near-IR (NIR) frequency combs [1, 3, 4, 5]. A large gap between initial NIR wavelengths and desired mid-IR (MIR) wavelengths results in low efficiency and high complexity of the overall system.

The major advantages of Cr:ZnS laser technology include ultrashort 2-cycle MIR pulses with a long central wavelength (2.4 μm), ultra-low timing jitter and relative intensity noise, and record-breaking (now in excess of 10%) efficiency of down-conversion from 2.4 μm to the longwave IR (LWIR) via optical rectification [8]. We leveraged these advantages tofR1 and fR2. The periodic interference beatings implement a compact, high-brightness, multi-octave, dual-frequency comb platform depicted in Fig.1. between the combs at the frequency ΔfR = fR1 – fR2 were detected with an MCT photodetector, digitized, and processed on a PC. The ΔfR was set to 125 Hz

Methods: The design of the frequency comb and its output spectrum are illustrated in Fig. 2. The Cr:ZnS laser oscillator is coupled to a supercontinuum generator. Here fs pulses are simultaneously amplified and compressed. The spectrum is broadened to a multi-octave continuum due to intrapulse three-wave mixing. Sub-fs-level timing jitter is realized by controlling oscillator pump power and cavity length. Further, the fundamental band of the comb (f) is coupled to a ZGP crystal that is arranged for the generation of the offset-free (0f) comb in the LWIR via optical rectification [8]. Remarkably, 0f and f optical frequency combs merge in a continuum, as illustrated in Fig. 2(b).

Results: To demonstrate the capabilities of our technology, we performed high-resolution DCS. The DCS experiments were carried out with two gases: N_2O at 100 mbar (0.04 volume mixing ratio in N2 buffer gas) and CO at 1000 mbar. We used 2 identical phase-locked combs at unequal repetition frequencies, fR1 and fR2. The periodic interference beatings between the combs at the frequency ΔfR = fR1 – fR2 were detected with an MCT photodetector, digitized, and processed on a PC. The ΔfR was set to 125 Hz for the 0f-band and to 54 Hz for the f-band, respectively. The obtained spectra (sampling interval of 80 MHz/0.0027 cm^{-1}) are in excellent agreement with the HITRAN simulations, as illustrated in Fig. 3.

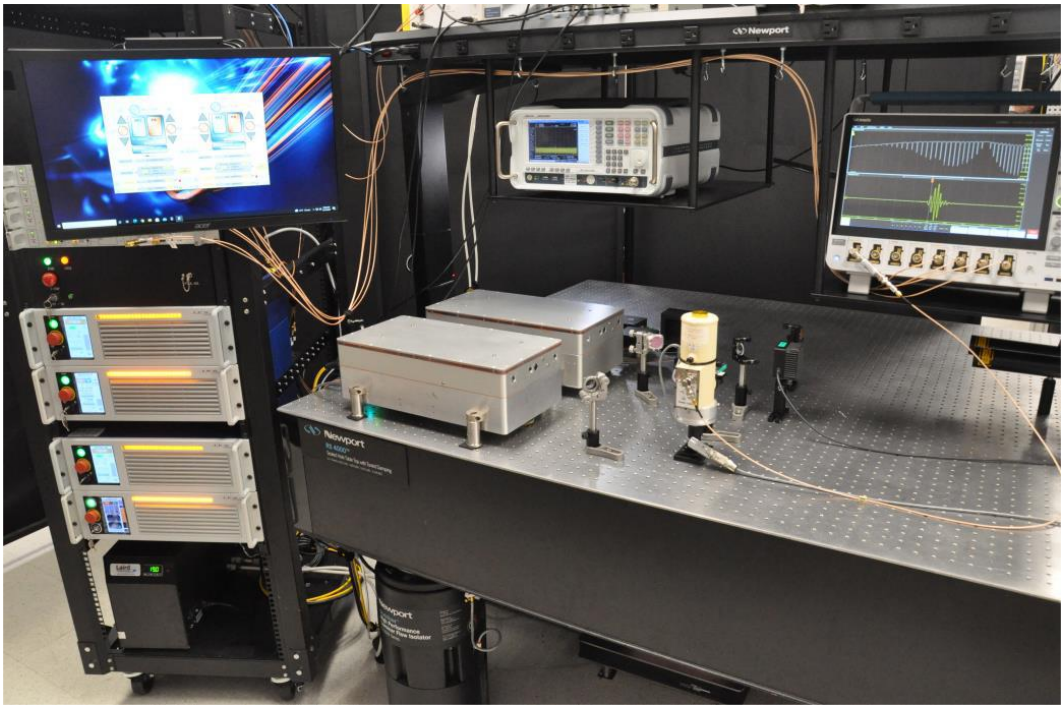

Fig. 1. Cr:ZnS Dual Comb Spectroscopy laboratory setup.

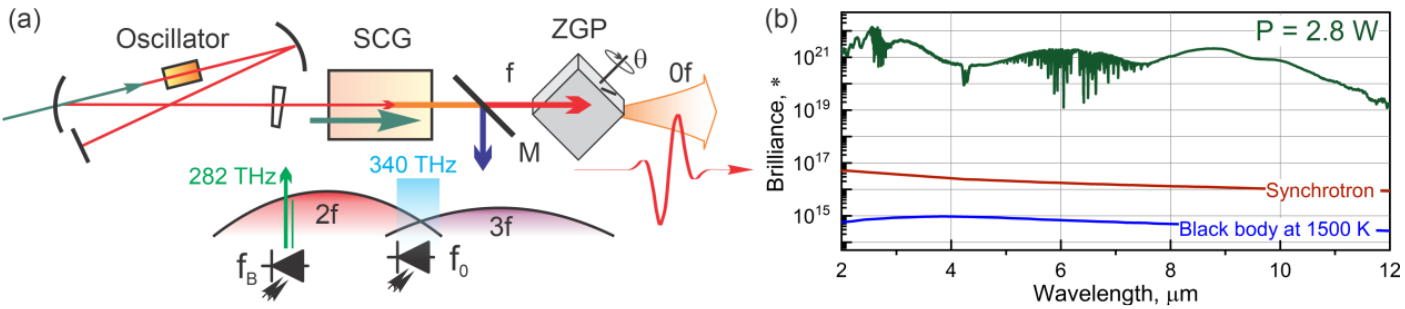

Fig. 2. (a) Cr:ZnS-ZGP frequency comb consisting of a fs laser oscillator, supercontinuum generator (SCG), and optical rectification stage based on a ZGP single crystal. The oscillator and the SCG are based on bulk polycrystalline Cr:ZnS and are optically pumped by CW EDFLS. The optical frequency comb is fully defined by the comb equation fn = f0 + n fR where there the radio frequencies f0 and fR are phase-locked to a Rb frequency standard and n is a large integer number. **(b)** The estimated brightness of the comb with average power P = 2.8 W and tooth spacing fR = 80 MHz is compared with the brightness of a synchrotron and a thermal source.

(*) the definition of the brightness in photons·(s·mm2·sr·0.1% BW)$^{-1}$ and reference spectra are reproduced from [1].

Conclusions: We report high-resolution gas-phase DCS with fully stabilized MIR frequency combs based on Cr:ZnS laser technology. The developed setup is compact, high power, and features very broad multi-octave spectral coverage in the MIR. This platform is very promising for the commercial realization of a non-targeted point-of-care breath analyzer.

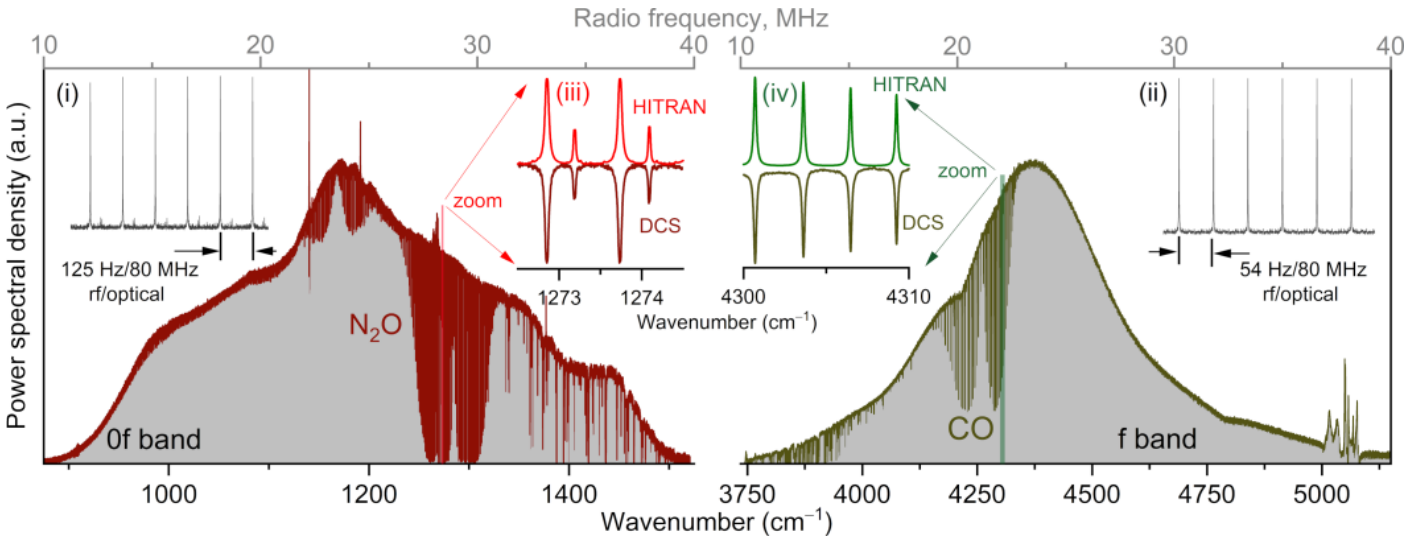

Fig. 3. *Results of DCS experiments: gray backgrounds and top axes, rf combs (Fourier transforms of time-series consisting of 0.5 GSamples acquired during 2 seconds); color lines and bottom axes, retrieved spectra (magnitudes of the comb's teeth mapped to optical domain). Inserts (i, ii) show the details of the frequency comb structures. Inserts (iii, iv) compare the details of retrieved spectra to HITRAN simulations. The spectrum in the 0f-band was measured with a Spectrogon LP6700 long-pass filter.*

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Madiha Malik Monday 13, Auditorium, 10h10

Department of Clinical Pharmacy, Pharmaceutical Institute, Kiel University, Germany

The potential of exhaled breath analysis in detection of SARS-CoV-2

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Background: Managing COVID 19 remains challenging due to unrestrained viable virus shedding, with symptomatic and asymptomatic patients being capable of transmitting SARS CoV-2 [1,2]. Recent studies have reported SARS CoV 2 to be mainly transmissible via droplets and/or aerosols [3,4]. Therefore, information on the dynamics of virus shedding through exhaled breath (EB) is essential. In this experimental study, we evaluated the SARS CoV 2 viral load and its progression in paired serial oronasopharyngeal swab (ONPS) and EB samples of COVID 19 patients [5].
Methods: We examined hospitalized patients initially tested positive for SARS-CoV-2. Paired ONPS and EB specimens were taken regularly on different days of hospitalization to analyse distinctive courses of infection. EB collection was performed through a simple, non-invasive method using an electret air filter-based device. SARS CoV-2 RNA was extracted from the filter and quantified by qRT-PCR.
Results: We provide a detailed evaluation of the time-dependent progression of the viral load of COVID-19 patients. Of 187 serial samples from 15 hospitalized patients, 87/87 ONPS and 70/100 EB specimens tested positive. Comparing the number of SARS-CoV-2 copies, the viral load of the ONPS was significantly higher (CI 99%, P<<0,001). The mean viral load per swab was 7.97 × 106 (1.65 × 102-1.40 × 108), whereas EB samples showed 2.47 × 103 (7.19 × 101-2.94 × 104) copies per 20 times exhaling. Unexpectedly, viral loads of paired ONPS and EB samples showed no correlation.
Conclusions: Assessing the infectiousness of COVID-19 patients merely through pharyngeal mucus might not be accurate. Exhaled breath could represent a more suitable matrix for evaluating infectiousness and might allow screening for non-, low- and superspreader-individuals and widespread variants. Further research on the viability of SARS CoV 2 in EB could remarkably minimize infection risks and improve understanding the dynamics of virus shedding.

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Miloš Selaković Monday 13, Room 27, 10h10

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RT-PCR Diagnosis of COVID-19 from exhaled breath condensate: wild-type and Delta variant

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Background: Laser-absorption spectroscopy is a promising alternative to mass spectrometry in future point-of-care diagnostics based on human breath that requires small and low-cost instrumentation. Its key characteristics are a fast and quantitative response [1] and potential for miniaturisation.
In this work, we present the development and characterisation of a compact mid-IR analyser for high-precision and simultaneous measurement of small volatile organic compounds (VOCs) [2].
Methods: The analyser uses an electrically tunable quantum-cascade laser [3] coupled to a 76-m-optical-path multi-pass cell. By means of the Vernier effect, the laser can be operated in six different spectral windows (each ~1.5 cm−1 wide) distributed between ~1163 cm−1 and ~1102 cm−1.
Measurements were performed in a flow-through configuration and VOCs were analysed at low pressure (~50 mbar) to minimize broadening of spectral lines.
The spectra were obtained at high resolution (<0.005 cm−1), which is not available in literature for most VOCs. Thus, we created our own reference database and developed a concentration-retrieval algorithm.
Results: Spectral screening of various VOCs reveals surprising and significant fine structure in the ro-vibrational spectrum of molecules containing up to four carbon atoms (C4). Such distinct narrow features were also observed for larger molecules (~C6) with rigid molecular structure or high-order symmetry. This raises new possibilities for laser-spectroscopic analysis of VOCs.
Our instrument is well-suited for the detection of small oxygen-containing VOCs at amount fractions down to ppb. We have achieved a typical precision of ~1 ppb for 25 s averaging time, demonstrated for methanol at an amount fraction of 10 ppm, and a large linearity range over 4 orders of magnitude.
Excellent selectivity of the method is achieved thanks to the unique spectral fingerprints of the investigated VOCs, enabling multi-compound measurements, even in a presence of water vapour and CO2, with an accuracy of <1%.
Conclusions: We developed a laser-based spectrometer suitable for fast, accurate, and precise detection of small oxygen-containing VOCs even in complex gas matrices. The system will address potential breath biomarkers in the framework of Zurich Exhalomics [4].

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Veronika Pospisilova Monday 13, Auditorium, 10h30

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Development of an Immediate Diagnosis of COVID-19 by means of on-line mass spectrometry

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Since the beginning of 2020, the world has been facing the devastating COVID-19 pandemic, with major impacts on public health and socio-economics. Mass diagnosis has been one of the cornerstones of the fight against SARS-CoV-2. The authorities have used mass screening to implement and adapt public health policies to limit the spread of the virus and its variants. The virological diagnosis is based on a 2-step process: first obtaining a biological sample and then performing a specific assay to detect the virus using molecular biology or antigenic test. Standard diagnostic could take up to several hours and rapid diagnosis assays (e.g. antigenic tests) suffer from very poor sensitivities, especially in asymptomatic patients.

Our study aims to develop a diagnostic method to screen the population via metabolite identification in the exhaled air. To do so, we used a newly developed proton transfer mass spectrometer (Vocus–PTR). This technology is based on a soft ionization system and could allow COVID diagnostic without performing invasive biological sampling while ensuring fast results (i.e., less than a minute). Hence, we performed two clinical studies in the summer of 2020 and spring of 2021. Altogether, 4 740 patients were recruited in Lyon University Hospital (n=121), and in a walk-through testing site (n=4 619), to be tested using the Vocus-PTR technique. All included patients were also tested with a RT-PCR assay for SARS-CoV-2, influenza and RSV detection.

The recruited population (47% women, between 6 to 95 years old) allowed a good technical performance evaluation. Among it, 531 patients were COVID positive by the RT-PCR method, with clinical status ranging from asymptomatic to acute respiratory distress syndrome (ARDS). Our results

demonstrate the usefulness, relevance, and ease-of-use of our methodology, but also highlight the challenges met to elaborate a diagnostic method in the general and hospitalized population.

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Gunnar Johanson Monday 13, Room 27, 10h30

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Diagnosis of acute cyanide intoxication among fire victims by breath analysis

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Background: Hydrogen cyanide (HCN) is an important cause of death among fire victims [1], as it is formed in abundance in modern buildings set on fire due to the presence of nitrogen-containing materials such as polyurethane foam in furniture. It is important to distinguish intoxication by HCN from that by carbon monoxide (CO), which is also formed in the fires, as the two require different treatments. There are currently no rapid diagnostic methods for HCN intoxication. Meanwhile, breath analysis seems to be an attractive, non-invasive option. We carried out four studies summarized herein [1-4], resulting in a PhD thesis [5].

Methods and results: Ongoing exposure to HCN vapor will obviously result in high levels of HCN in exhaled breath, regardless of the systemic dose. We studied the washout of HCN in the breath from 10 volunteers following a brief experimental exposure at a dose comparable to that from one cigarette. The post-exposure decline in breath was fast, the average half-time being 16 s [2].

HCN may be produced by bacteria present in the mouth cavity. We measured the background level in breath from 40 healthy, adult non-exposed subjects by near infrared cavity-ringdown spectroscopy. The median was 4.4 ppb [3].

We developed a PBPK-PD model and simulated 1 h of exposure at 100 ppm HCN, a life-threatening dose. The high initial breath level seen immediately after exposure dropped to about 1 ppm within a minute, due to rapid washout from the airways. Thereafter, HCN in breath remained stable (decreasing to 0.5 ppm in 7 h) reflecting the systemic dose [6].

Conclusions: Our model simulations suggest far higher levels of HCN in breath from intoxicated fire victims than measured in non-exposed subjects. Confounding by ongoing inhalation of HCN is not a problem as long as the person has been breathing fresh air for a minute. Our studies confirmed that, in theory, breath monitoring is useful method. The remaining problem at the time was that there was no suitable, rapid and sufficiently sensitive method available for field use. Today, with the rapid development of handheld detectors, the situation may be different.

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Renelle Myers Monday 13, Auditorium, 11h20

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Breath Testing for mild SARS CoV-2 infection

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Background: The SARS CoV-2 pandemic is here to stay, and the world needs to prepare for additional waves from new variants and seasonal recurrences. The greatest risk to public health is transmission from people with mild respiratory symptoms. A rapid and accurate point-of-care screening test is needed to identify SARS CoV–2 infections with mild symptoms and differentiate from other, less virulent, viral respiratory tract infections. The aim of this study was to develop a breath-test to identify people with SARS CoV–2 infection when they have mild respiratory symptoms.
Methods: A prospective, real-world, observational study, collected breath samples from ambulatory patients with mild symptoms presenting to a community test site for RT-PCR testing from Sept. 2020 until Feb. 2022, spanning the emergence of Alpha, Beta, Gamma, Delta and Omicron variants. A subset of patients underwent follow-up breath testing at 8 – 12 weeks post diagnosis as well, to control for biological variability. Breath samples were collected into Tedlar Bags, transferred to an adsorbent trap, and analysed with thermal desorption-gas chromatography-mass spectrometry.
Results: 132 breath samples were analysed from 50 RT-PCR +ve, 60 RT-PCR –ve and 22 repeat tests 8 to 12 weeks following a +ve RT–PCR test. Multivariate analysis identified 13 features separating SARS–CoV 2 +ve and -ve with a sensitivity of 82%, specificity of 65% and an AUROC of 0.762. 13 molecular features were also found to be significantly different in the 22 repeat samples compared to the initial 50 samples from SARS CoV-2 +ve patients, despite many having persistent mild symptoms. Seven differentiating features were common between the follow up and initial +ve samples.
Conclusions: Exhaled breath is a promising tool to identify individuals with mild respiratory symptoms due to different variants of SARS CoV-2 infection from those with symptoms due to other viral infections. Significant breath biochemisrty differences appear to be present at 8-12 weeks following infection.

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Ines Weber Monday 13, Room 27, 11h20

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Metabolic health monitoring through breath acetone detection with compact sensors

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Background: A major concern of today's society is metabolic health, particularly obesity, which is related to several diseases (e.g., diabetes) [1]. Urgently needed are tools to track fat-burn rates in individuals. This can be done through non-invasive acetone detection, a metabolic marker of lipolysis [2]. Specifically, acetone is formed during hepatic β-oxidation of fatty acids that further divide into acetoacetate. The latter undergoes decarboxylation and enzymatic degradation to acetone [3], which is volatile and measurable in exhaled breath. Therefore, portable gas sensors are needed that can routinely measure breath acetone to guide therapeutic actions (e.g., dieting and exercise) and improve their effectiveness. Most promising are metal oxide sensors that are highly sensitive and, through the combination with catalytic filters [4], highly selective.

Methods: The compact acetone detector consisted of a Pt/Al_2O_3 nanoparticle filter pre-screening a Si/WO_3 sensor [5]. First, this detector was evaluated in laboratory conditions to assess its sensitivity and selectivity [6]. Thereafter, it was tested on the exhaled breath of nine volunteers.[7] All volunteers performed a cardiorespiratory fitness-adapted submaximal aerobic exercise protocol[8] followed by a subsequent rest. Breath was sampled every 5 min during exercise, as well as every 30 min during 3 hours of post-exercise rest with an end-tidal breath sampler[9] and analyzed by mass spectrometry and with the Pt/Al_2O_3 filter-enhanced Si/WO_3 sensor.

Results & Conclusions: Figure 1 shows the acetone concentrations during exercise ($0 \leq t < 60$ min) and rest ($60 < t \leq 240$ min) as determined with mass spectrometry (triangles) and the Pt/Al_2O_3 - Si/WO_3 sensor (circles) exemplarily for one volunteer. The steady increase in acetone indicates enhanced lipolysis, as confirmed also by blood measurements. Most impressively, the detector exhibits high accuracy (i.e., 25 ppb for all 9 volunteers) and is robust also against interferants such as isoprene that spikes at the onset of exercise [10] as well as orders of magnitude higher ethanol concentrations from hand disinfection in the same room. Currently, this detector is used in a randomized clinical trial study at the University Hospital Zurich to monitor intermittent fasting diets in 72 volunteers.

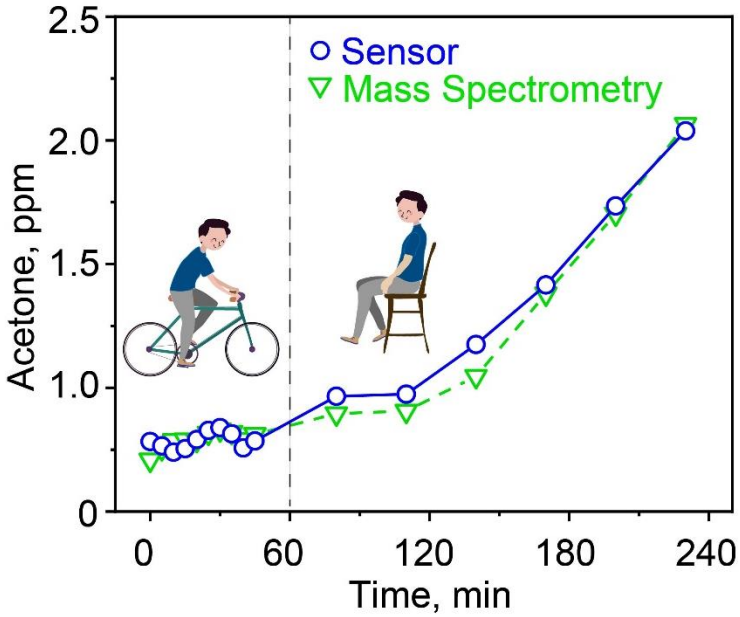

Fig. 1. End-tidal acetone increases during exercise and rest, indicating enhanced lipolysis, as detected by the compact sensing device (circles) and mass spectrometry (triangles).

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Amalia Berna Monday 13, Auditorium, 11h40

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Discovery and clinical validation of breath biomarkers of SARS-CoV-2 infection in children

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Background: COVID-19, caused by infection with SARS-CoV-2, continues to be a major global health concern, with recurring waves of infection even in well-resourced countries with moderate-to-high immunization rates. While vaccination is highly effective against severe disease and death, it does not produce "sterilizing" immunity, and efficacy is reduced in the elderly/immunocompromised. Additionally, vaccine hesitancy remains an unsolved challenge. In addition, children have been consistently undertested for SARS-CoV-2, in part due to increased asymptomatic and mild infections (1). Asymptomatic infection in children may facilitate viral transmission (2). A rapid, inexpensive, simple, and non-invasive method to detect pediatric SARS-CoV2 infection would have a major impact on the COVID-19 pandemic.

Methods: In spring 2020, we sought to determine whether SARS-CoV-2 infection might likewise be associated with changes in breath VOCs. We enrolled two independent cohorts of healthy controls and SARS-CoV-2-infected children admitted to the Children's Hospital of Philadelphia (CHOP) (3). Breath samples were collected from children (4-20 y) and analyzed through state-of-the-art GCxGC-ToF-MS.

Results: From 84 targeted VOCs, we identified 6 biomarkers that are significantly and reproducibly increased in abundance in the breath of children infected with SARS-CoV-2. Of these biomarkers, several aldehydes (octanal, nonanal, and heptanal) drew special attention, as aldehydes are also elevated in the breath of adults with COVID-19 (4). These VOCs are similar to those formed during lipid peroxidation of cells infected with other respiratory viruses, including influenza (5, 6). Using the sum of the abundances of these 6 biomarkers ("cumulative abundance") as a diagnostic strategy, our biomarkers yield a sensitivity of 91% and specificity of 75% for diagnosis of pediatric SARS-CoV-2.

Conclusions: Our key proof-of-concept pilot study at CHOP nominated candidate biomarkers that characterize the exhaled breath of SARS-CoV-2-diagnosed children and validated their accuracy in a second cohort. To characterize the specificity of these biomarkers, we are currently evaluating the breath volatile profiles of children with symptomatic or asymptomatic SARS-CoV-2 infection, compared to children with other upper respiratory tract infections.

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Andreas T. Güntner Monday 13, Room 27, 11h40

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Screening methanol poisoning with a portable breath detector

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Methanol poisoning outbreaks after consumption of adulterated alcohol frequently overwhelm health care facilities in developing countries. Here, we present how a recently developed low-cost and handheld breath detector can serve as a non-invasive and rapid diagnostic tool for methanol poisoning [1]. The detector combines a separation column and a micromachined chemoresistive gas sensor fully integrated into a device that communicates wirelessly with a smartphone. The performance of the detector is validated with methanol-spiked breath of 20 volunteers (105 breath samples) after consumption of alcoholic beverages. Breath methanol concentrations were quantified accurately within 2 min in the full breath-relevant range (10 – 1000 ppm) in excellent agreement (R^2 = 0.966) with benchtop mass spectrometry (PTR-TOF-MS). Bland-Altman analysis revealed sufficient limits of agreement (95% confidence intervals), promising to indicate reliably the clinical need for antidote and hemodialysis treatment. This simple-in-use detector features high diagnostic capability for accurate measurement of methanol in spiked breath, promising for rapid screening of methanol poisoning and assessment of severity. It can be applied readily by first responders to distinguish methanol from ethanol poisoning and monitor in real time the subsequent hospital treatment.

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Nicholas Kenyon Monday 13, Auditorium, 12h00

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Markers of SARS-CoV-2 Infection in exhaled breath condensate

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Background: Exhaled breath condensate (EBC) has been increasingly used as a novel method to study airway inflammation for a variety of pulmonary diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease, and interstitial lung diseases. Furthermore, metabolomic patterns in EBC have been found to differ between healthy, inflammatory, and infectious airways. In this study, we aim to determine whether EBC contains metabolites that are diagnostic of COVID-19. Furthermore, we investigate whether the inflammatory metabolites found in EBC correlate with COVID-19 disease severity and prognosis.

Methods: EBC samples were collected from adults with confirmed COVID-19 disease alongside control subjects using a previously reported collection tool [1]. Collection occurred at the UC Davis Medical Center under an IRB-approved protocol, #1636182. Demographic information about volunteers was collected through a questionnaire and review of electronic medical records. Samples were processed under a newly confirmed technique to deactivate any potential SARS-CoV-2 virus in condensate samples [2]. EBC samples underwent liquid chromatography-quadrupole time of flight mass spectrometry (LC-qTOF) analysis. The dataset was randomly split into a training and validation set for machine learning algorithms to identify the COVID-19 breath signature.

Results, Conclusion: At time of abstract submission, sample collection and chemical analysis are ongoing, with n=59 total EBC samples collected. During the IABR Meeting, the authors will present the latest findings, detailing the accuracy, sensitivity and specificty to diagnose SARS-CoV-2 from exhaled breath condensate. We will present findings on whether COVID-19 biomarkers correlated with disease severeness and symptoms.

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Alexander Pospelov Monday 13, Room 27, 12h00

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New express method for real-time breath analysis with quantum point-contact sensors

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Significant progress in the development of noninvasive diagnostic tools based on breath analysis can be expected if one employs a real-time detection method based on finding a spectral breath profile that would contain some energy characteristics of the analyzed gas mixture. Using the fundamental energy parameters of a quantum system, it is possible to determine with a high accuracy its quantitative and qualitative composition. Among the most efficient tools to measure energy characteristics of quantum systems are sensors based on Yanson point contacts [1]. The quantum properties of point-contact sensors make it possible to record the spectral profiles of the breath which contain comprehensive information about this complex gas medium [2]. This allows us to propose and develop the point-contact method for spectral analysis of complex gas mixtures, which does not require the detection of separate components of the analyzed gaseous medium [3]. It was successfully applied to analyze point-contact breath profiles and accurately find concentrations in the human blood of such hormones as serotonin and cortisol, the hormones of "happiness" and "stress", which are important for medical diagnosis of various states of the human organism, including psycho-emotional states. This method allowed us to find the sections of maximum correlation in the breath spectra between the hormone concentration in the human blood and the values of the average voltage of the point-contact sensor response curve and determine analytical expressions for relevant calculations.

For the first time, it became possible to analyze the human hormonal background using the most convenient biological material – the breath medium. With the hormones serotonin and cortisol used as an example, it is shown that concentrations of these substances can be monitored in real time, while in the case of conventional medical analysis it takes hours and even days. This approach significantly increases the effectiveness of preventive and therapeutic procedures.

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Laura Miles Monday 13, Auditorium, 12h20

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Breathing new life into data quality

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Breath analysis in disease diagnosis analyses vapour-phase organic compounds which are by-products of metabolic processes in the human body. Breath sample collection is non-invasive, and enables additional research into diagnosis of respiratory and gastrointestinal diseases among others. If accepted into mainstream clinical diagnosis then sampling would be performed over numerous locations, with multiple samples per patient potentially over time spans covering months or even years. This presentation shows how thermal desorption can utilise some of its key features to resolve these challenges and ensure good data quality across extended timeframes as the gold standard of breath analysis. Sample collection onto sorbent tubes is straightforward, and simple barcodes enable enhanced sample tracking. The tubes are also easy to transport and store due to their small size which is key for the thousands of samples that could be expected in a full clinical trial.

Management of a large sample cohort and achieving good quality results with confidence results in an analytical challenge for a number of reasons:

- Sample tracking and security – chain of custody style management is the optimum solution by using electronic sample tracking. Samples are invaluable as they often cannot be replicated, options for archiving remaining sample after analysis is very important.
- Sample integrity – once a sample is taken it must be transported and stored under optimum conditions in order to maintain the quality of the sample
- Standardisation – procedures for sampling and analysis will need to be drafted to take into account quality control checks at various stages for increased confidence over the lengthy clinical studies expected
- Inherent sample features - breath by nature is saturated with water vapour and contains compounds of interest at trace levels, use of pre-concentration and water management stages are essential for successful analysis

Standardisation can be achieved with multiple standard options including automated internal standard addition for tracking responses over longer time periods such as a clinical trial. Lastly system features such a dry purging and sample stacking enable some of the inherent features of breath to be managed improving reliability of identified compounds with GC-MS analysis and therefore successful analysis. In summary, TD-GC-MS provides researchers of breath with resolutions to common analytical challenges and improvements to reliable workflows for good data quality.

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Gennadii Kamarchuk Monday 13, Room 27, 12h20

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Quantum point-contact sensors: new mechanisms and concepts for real-time breath analysis

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Point-contact sensors are a new type of modern transducers. The principle of their operation is based on the unique physical properties of Yanson point contacts, which make them sensitive to the action of gases and liquids [1]. A major characteristic feature of the Yanson point contact is that it is a nanostructure whose electronic states can be directly controlled with high precision through its conduction properties – quantized conductance in the first place.

The quantum mechanism of selective detection in gaseous and liquid media discovered by us [2] is a direct demonstration of the broad spectrum of opportunities Yanson point contacts have brought into the sensor activities. The quantum properties of point-contact sensors make it possible to record the spectral profiles of the breath, which contain comprehensive information about this complex gas medium. The point-contact sensor profile of breath has a nonmonotonic spectral character with a number of nonlinearities, maxima, and minima. Unlike other nanosensors and conventional sensors based on the principle of changing electrical conductance, point-contact sensors are able to display energy interactions and their features explicitly in the breath profile. It led to the possibility of simple and effective noninvasive medical diagnosis based on real-time analysis of patients' breath. This possibility was successfully demonstrated by the first evidence for the detection of virulent strains of Helicobacter pylori infection in real-time using the point-contact spectroscopic concept of breath analysis [3]. The spectral approach to detecting complex gaseous media allowed developing new methods to accurately find concentrations in the human blood of such hormones as serotonin, melatonin, and cortisol [4, 5], which are important for medical diagnosis of various states of the human organism, including psycho-emotional states.

Our studies testify that point-contact sensors demonstrate the wide arsenal of opportunities and the broad prospects for the sensor breath tests to be used for detecting pathological changes in the human body at the earliest stages of development right up to early warning of these changes.

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Rosamaria Capuano Monday 13, Room 27, 12h40

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Colorectal cancer detection by breath analysis using a gas sensor array. A preliminary study

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Background: Colorectal cancer (CRC) is the 3rd cause of death in Europe [1]. Colonoscopy is considered the gold standard as a diagnostic tool. However, it is invasive and uncomfortable. The analysis of exhaled volatile organic compounds (VOCs) is a promising alternative as a non-invasive tool for detecting several cancers, including CRC [2]. Sensor arrays play an essential role in breath analysis because they can provide quick analysis times and ease of use than more complex analytical instruments. Promising results have been reported in the literature about using gas sensor arrays to detect CRC by breath analysis VOCs [3]. In this paper, a sensor array has been applied to discriminate the breath of CRC affected patients and healthy subjects. The sensors, made of porphyrinoids coated quartz microbalances (QMBs) [4], have been previously applied to colon cancer in a murine model in particular to investigate the role of LOX-1 (a primary receptor of oxidised- LDL) in tumorigenesis and metastatic processes [5].
Methods: Due to COVID-19, the original experimental plan was halted. Thus, here preliminary data related to three patients and fourteen are reported. Subjects wore a nose clip and exhaled deeply to total lung capacity into a collecting system composed of a mouthpiece connected to a T-valve that allows directing the first portion of exhaled air in a waste bag (200 ml) and the remaining part in a sampling bag (3l).
Results: Linear Discriminant Analysis (LDA) has been applied to the collected data to estimate the sensor array's discrimination capabilities. The first canonical variable allows good discrimination between CRC tumour patients and the control group ($p<0.001$).
Conclusions: Preliminary results show that an electronic nose based on porphyrinoids coated QMBs might discriminate the breath of CRC-affected patients regarding healthy subjects.

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Jens Herbig Monday 13, Auditorium, 14h30

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Variability of breath-borne volatiles – Curse or blessing?

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Background: Breath gas analysis promises non-invasive diagnosis after exhaling into a breath analyser. This vision is coined by existing tests where one blood sample, or one X-ray is analysed, or your DNA is once sequenced. But does a single breath screening provide the real picture? For some compounds we already know that their exhaled concentration can change rapidly. In this talk, we will discuss the "volatile" nature of breath, an allusion to both the volatile nature of the compounds as well as the variability of their concentrations.

Methods: We have studied the breath of a subject over the course 12 hours, taking samples every 10 minutes. Each data point consists of three exhalations (within 2 minutes). Ambient VOC concentrations are automatically recorded in-between sampling points. We have allowed - and documented - all influences that occur in a realistic setting, such as the ingestion of food and beverages, variation of the room-air and ventilation.

For the analysis, we employed a real-time trace gas analyser, a PTR-TOF 1000 in combination with a buffered end-tidal (BET) breath sampling system [1].

Results: We study the measured variability in a person's breath spectrum for all compounds observed. By comparing short- and long-term variability, we can separate the short-term influences of the analyser, sampling, and exhalation kinetics from the long-term variability, which can then be attributed to physiology.

Conclusions: Our data exemplifies the intra personal variability that has to be expected in exhaled concentrations. This variability must be assessed on a compound-by-compound basis before they can be proposed as biomarkers for screening. Breath applications that rely on monitoring observe and can account for this variability. We have also exemplified the influence of external factors, such as room-air and food intake, that need to be considered in a realistic scenario.

However, observed variations are not purely random, but suggest an underlying physiological process. In contrast to other bodily fluids, breath does allow for frequent sampling and the added dimension of time could open new possibilities for breath-borne volatiles that have previously been neglected as unspecific or too variable. Acetone is a first example that has a concentration within the range of portable sensors that brings such applications within reach.

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Agnieszka Smolinska Monday 13, Room 27, 14h30

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Non-invasive breath collection in murine models: an optimization and case study on abdominal sepsis

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Background: Abdominal sepsis is a severe condition that requires early and adequate treatment in order to improve patient outcomes. Volatile organic compounds (VOCs) in exhaled breath might have a potential to be used as a tool for diagnosing and monitoring abdominal sepsis. Although, exhaled breath is non-invasive, investigating abdominal sepsis in clinical settings might be challenging due to various factors. Therefore, small laboratory animal studies might contribute in obtaining fundamental insight in alterations in exhaled VOC composition.
Methods: A novel device was developed for non-invasive breath collection in mice using glass nose-only restrainers. First, C57Bl/6J healthy mice were used to test reproducibility of the sampling device. Secondly, upon optimization of the mice breath-sampling device, it was used to investigated murine model of abdominal sepsis using Escherichiae Coli and Enterococcus Faecalis as inducers. Exhaled air was collected on desorption tubes and analysed for VOCs by gas chromatography time-of-flight mass spectrometry.
Results: The mice-breath sampling device showed the best reproducibility using an air flow of 185 ml/min and a collection time of 20 minutes. The exhaled breath samples of mice were easily distinguished from the blanks samples. The case study showed a specific breath profile for animals developing Escherichiae Coli-induced and Enterococcus Faecalis-induced abdominal sepsis. Moreover, the time course of the abdominal sepsis development was observed.
Conclusions: The mice breath-sampling device shown here has the potential to facilitate VOC research in relation to disturbed metabolism and or disease pathways. The mice-VOC analysis demonstrated the ability to distinguish between Escherichiae Coli-induced and Enterococcus Faecalis-induced abdominal sepsis, thereby offering unique opportunity to study the course of abdominal sepsis.

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Sean Harshmann

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Monday 13, Auditorium, 14h50

A searchable food and drink related volatile organic compound library for exhaled breath contaminant determination

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Exhaled breath (EB) has had minimal success being adapted into a clinical setting due to several limitations [1]. For instance, diet-related volatiles can influence EB results [2,3]. While volatile profiles of many foods or drinks have been determined, often the results are scattered within the literature and not necessarily sampled utilizing similar specificity to common EB methodologies [4]. Here, a searchable database, associated with structures registered and curated in the EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard), has been created using both PTR-MS and TD-GC-MS from the headspace of food, drinks, and other contaminants with potential to impact EB research [5].

Test material was placed in a headspace vial. 200 PTR-MS scans were taken directly above the sample (Ionicon). TD-GC-MS samples were collected, from the headspace, using a custom 3D printed cap affixed to a mass flow controller (Markes International, ThermoScientific). Data were evaluated for features representing the top 25% of overall signal in each data set (PTR & TD-GC-MS) for database inclusion. Furthermore, several food and drinks were ingested by participants (n=5) and lower airway exhaled breath was collected utilizing both PTR-MS and TD-GC-MS.

To date, the database consists of 40+ food, drink, and other exogenous contaminants. The data collected showed a robust difference in the volatile profile among different substances. For example, ripening bananas show a complex volatile profile as the aging process proceeds. Furthermore, many food or drinks have volatile components that overlap with commonly identified EB components. For instance, the headspace above bananas contains ethanol and ethyl acetate, compounds identified in previous EB studies [6]. Ingestion experiments utilizing bananas showed an 11.1 and 48.7 fold increase for ethanol and ethyl acetate, respectively, at two minutes post ingestion. By 15 min. post ingestion, a 2.7 and 1.5 fold increase in these compounds was observed.

A database of exogenous contaminant volatile profiles was created for searching EB data for plausible contamination. These data will allow for identification of expected volatiles, corresponding exogenous sources to ultimately establish a standard fasting time. Abstract does not reflect EPA policy.

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Antao Gao Monday 13, Room 27, 14h50

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Identification of *Burkholderia pseudomallei* infection using patient breath

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Background: Melioidosis is an emerging life-threatening tropical infection caused by the bacterium *Burkholderia pseudomallei* (Bp)1,2, which is endemic in approximately 46 countries with a high fatality rate.3 The key factors to limit fatalities are a quick diagnosis and appropriate antimicrobial treatments.1,2,4 Melioidosis diagnosis is challenging due to its divergent clinical manifestations, the paucity of conventional identification methods, and the time-consuming culture-based procedures.4,5 The profiling of volatile molecules, either microbially-derived or associated with the host-pathogen interaction, is a novel approach, we propose as a potential use for rapid, non-invasive infection detection.6,7 We hypothesize that during a melioidosis infection there will be volatile compounds in the patient's breath that will be sensitive and specific to infection etiology.
Methods: In this proof-of-concept study, we collected breath samples from patients infected with Bp (Bp+) as well as patients with other infections (Bp-) as controls to test this hypothesis. We also tracked additional Bp+ patients on treatment with longitudinal samples over 28 days. We collected breath onto 3-bed thermal desorption tubes and analyzed using comprehensive two-dimensional gas chromatography time of flight mass spectrometry (2D GC×GC-TOFMS). The resulting data was preprocessed and then subjected to statistical analysis, including unsupervised models (PCA, hieratical clustering, and tSNE) and the Boruta algorithm, as a supervised feature selection method.8
Results: Through data analysis, we putatively identified 11 volatile molecules that could clearly discriminate between Bp+ (n=6) and Bp- (n=7) samples. The selected set of biomarkers was applied for the prediction of the disease status of seven additional patients and all samples were predicted correctly. Moreover, monitoring the relative concentrations of these biomarkers among longitudinal samples collected from Bp+ patients revealed statistically significant variation patterns in the abundances of the biomarkers during the treatment period.
Conclusions: The prospective validation of these biomarkers in a larger cohort could yield clinical implementation as a non-invasive and timely diagnostic test for infections with this emerging pathogen.

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Y. Lan Pham Monday 13, Auditorium, 15h10

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Uptake and emissions of volatiles from materials used in-line during breath sampling

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Sampling approaches for breath analysis require the use of suitable parts that interface the breath donor (e.g., patient) with the breath collection system. The primary requirement of this interface is to ensure patient safety by introducing a sterile barrier that reduces the risk of transmission of pathogens between patients. Further considerations for suitable interface materials are that they exhibit low emissions of volatiles to reduce health risks to patients through inhalation exposure as well as confounding factors in biomarker datasets [1], and that the adsorption of breath volatiles is avoided in order to retain sample authenticity.
To address these issues, several materials used in conjunction with the commercial respiration collector for in-vitro analysis (ReCIVA, Owlstone Medical) device were examined. Besides the silicon facemask commonly used with the ReCIVA system, a low-cost and reusable 3D-printed mouthpiece adapter was developed for use with a pulmonary function filter for sampling breath using the same device [2,3]. The present study compared volatile emissions from adapters printed from polymer resin materials, a pulmonary function filter, and the commercial silicon facemasks. Further, the uptake through adsorption onto the material surfaces of a variety of representative compounds commonly present in the human breathome was investigated to ascertain potential losses in sample composition. All analyses were performed in tandem using proton-transfer-reaction and comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (PTR-TOFMS and GCxGC-TOFMS, respectively).
This presentation reports on the degree of emissions and adsorption of the aforementioned breath sampling interfaces and highlights the influence of different material treatment procedures on the extent of background volatiles emitted from such parts. Overall, the choice and treatment of sampling materials represents an important consideration in quality assurance.

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Patricia Fuchs Monday 13, Room 27, 15h10

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Monitoring of VOC-profiles during *Streptococcus suis* infection in pigs

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Background: *Streptococcus suis* is one of the five most important porcine pathogen worldwide, that causes a wide range of clinical diseases in swine with major economic losses. Due to its zoonotic pathogenicity *S. suis* can be transmitted to humans and has various clinical manifestations. Confirmation of infection is achieved by well established procedures but their technological limitations and complexities may lead to delays in diagnosis. Recent studies suggest that bacterial infections could be reflected through exhaled VOC profiles non-invasively.

Methods: In this in vivo study breath gas samples were taken from 11 spontaneously breathing pigs during a complete *S. suis* serotype 2 infection cycle at 11 time points. Breath sampling was carried out under high safety conditions with a sterile glass syringe in the alveolar phase of expiration after placing the pigs in a canvas sling. VOCs were preconcentrated onto triple bed needle trap devices (NTDs), thermally desorbed in the GC inlay and analyzed by GC-MS. In parallel, the headspace of feces samples was analyzed by SPME-GC-MS.

Results: The GC-MS assays provided reliable quantification of exhaled and headspace VOC profiles down to pptV level. During the monitoring process significant differences in 8 volatile organics (such as aldehydes, ketones, carboxylic acids and aromatic compounds) were detected between infected and non-infected animals. Exhaled VOCs showed significant changes linked to inflammation or bacterial metabolism. Changes in VOC concentrations over feces can be correlated to modifications in pigs gut microbiome.

Conclusions: Results underline the potential of VOC analysis for the monitoring of infectious diseases in vivo. As a quick and completely non-invasive method dynamic VOC profiling may add comprehensive information on infection processes.

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Karl Unterkofler Monday 13, Auditorium, 15h30

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Understanding patterns and variations in breath gas concentrations – what we can learn from modeling

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Background: Researchers looking for biomarkers often search for specific patterns of volatile organic compounds (VOCs) from different sources such as breath, urine, or blood. However, they are seldom aware that these patterns change depending on the source they use. We present a simple model to demonstrate that the distribution patterns of VOCs in blood fat, mixed venous blood, alveolar air, and end-tidal breath are different.

Methods: To show that concentration pattern of VOCs differ substantially when investigating different body fluids we used a simple model that consisted of two body compartments only; namely a fat compartment and a residual body compartment. The reason for this choice is the availability of the blood:fat partition coefficients for VOCs with very different blood:air partition coefficients. We assumed a uniform distribution of the VOCs in end-tidal breath and computed the corresponding distribution in alveolar air, arterial blood, mixed venous blood and fat.

Results: To illustrate how a particular VOC profile changes during the transfer of VOCs between different tissues, fluids and excretions, 16 volatiles were selected. Although the main selection criterion was the availability of the experimentally determined values of blood:air and blood:fat partition coefficients, an effort was made to include species exhibiting a wide range of blood:air partition coefficient values covering a range from 0.42-1500. Figure 1 shows that the concentration ratio between alveolar and end-

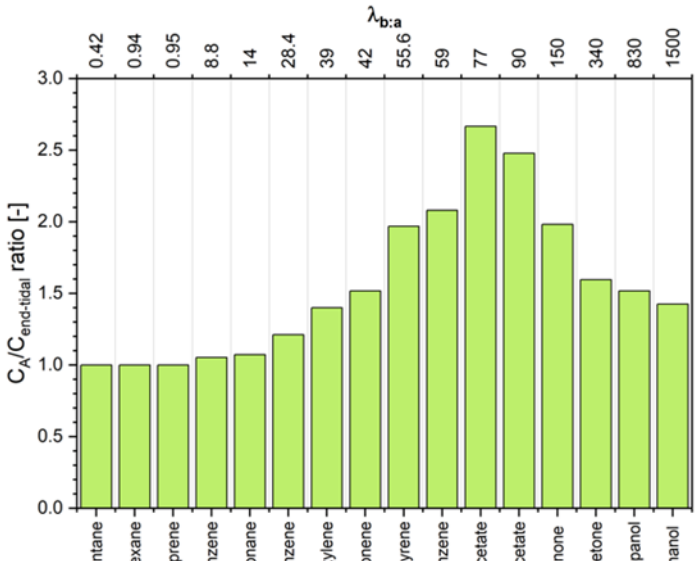

Fig. 1. Concentration ratio between alveolar C_A and end-tidal air $C_{end\text{-}tidal}$ for VOCs under scrutiny. Compounds are ordered with respect to increasing blood:air partition coefficient.

tidal air of VOCs is not constant and depends on the blood:air partition coefficient. The distribution of these VOCS in arterial blood, mixed venous blood, and fat tissue yields also different pictures, when assuming a uniform distribution of these VOCs in end-tidal breath.
Conclusions: If we assume a uniform distribution of VOCs in end-tidal breath, we will get a completely different picture in the blood of the fat and vice versa. Consequently, the involvement of different bodily fluids and secretions in biomarker discovery within the volatilomics can result in the identification of different sets of biomarkers related to the same disease as different matrices promote compounds with different physico-chemical features. For example, it could happen that some VOCs, which show up as significant biomarkers when looking at one matrix, might not even be detectable in other matrices due to limits of detection and vice versa. It also means that classification (e.g., disease / no-disease) algorithms trained on VOC data from one matrix cannot easily be transferred/generalized to other matrices.

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Nele Kemnitz Monday 13, Room 27, 15h30

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Mass spectrometric breath screening of patients with pulmonary bacterial infections

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Background: Pulmonary bacterial infections represent a common problem in clinical medicine. The most frequent bacterial pathogens in respiratory tract infections are Streptococcus pneumoniae and Haemophilus influenzae. In recent years identification of bacteria by their volatilome gained interest as it holds the promise of a non-invasive early pathogen detection. In this study, we investigated exhaled volatile organic compounds (VOCs) as potential non-invasive markers for bacterial presence and host response in a large non-preselected cohort.

Methods: 554 adult patients were screened for 26 common respiratory pathogens in a clinical test center. Breath VOCs were measured continuously in real-time via a high-resolution proton transfer reaction time of flight-mass spectrometry (PTR-ToF-MS). Detection and identification of pathogens were done by using a standardized multiplex-PCR.

Results: Out of the 554 subjects, 417 did not have an infection, 97 tested positive for H. influenza, 40 tested positive for S. pneumoniae. In the breath samples, 46 potentially blood borne substances could be detected in low ppbV concentrations. Significant differences in VOC profiles were detected between healthy and H. influenzae infected subjects and between H. influenzae/S. pneumoniae infected subjects with symptoms compared to H. influenzae/S. pneumoniae infected subjects without symptoms. In total seven compounds showed significant changes in VOC concentration. These substances can be linked to bacterial metabolism or are associated with inflammation and oxidative stress.

Conclusions: This study shows that exhaled VOC profiles may mirror bacterial presence and host response. In a perspective, breath analysis could deliver comprehensive real-time information in addition to established methods.

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Kavita Jeerage Monday 13, Auditorium, 15h50

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Multicomponent gas standards for breath biomarker analysis

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Primary standards ensure the accuracy of gas and solution-based calibration standards as well as absorbance and transmittance scales for chemical spectrophotometers. These standards, in turn, support chemical manufacturing, pharmaceutical production, and clinical measurements. While numerous serum-based reference materials are available to ensure the quality of blood measurements, analogous materials for forensic or clinical breath measurements are limited. Forensic breath analysis is dominated by the alcohol breathalyzer. Humidifed calibration gases can be generated from ethanol-water solutions supplied as Certified Reference Materials (CRMs) by National Metrology Institutes (NMIs) or by solutions traceable to primary standards. Non-humidifed or “dry gas” standards, easily deployed in the field, are functionally equivalent for evidentiary analyzers [1]. Dry gas standards are also employed to calibrate analyzers for hydrogen and methane or nitric oxide. During intoxication, ethanol is approximately two orders of magnitude higher than endogenous alcohols or acetone and interference is rarely observed [2]. Future breath tests are likely to contend with interference due to lower concentrations of clinical biomarkers. For example, gastrointestinal disorders lead to ppm increases in hydrogen or methane [3] and nitric oxide remains below 100 ppb in asthmatic patients [4]. Gas standard(s) containing the major constituents of breath could be used for calibration and to check for cross sensitivities. In this presentation, we discuss the history of gas-based reference materials developed to ensure reliable atmospheric measurements, the evolution of multicomponent gas standards, and some of the challenges. Developing a reference material to support clinical breath measurements may present similar challenges due to the chemical classes involved. We end with a discussion of scenarios where specialized analytical instruments may not require gas standards.

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Waqar Ahmed Monday 13, Room 27, 15h50

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Targeting microbial volatiles as biomarkers of lung infection in the ICU

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Background: Microbial identification is pivotal in guiding choice of treatment of intensive care patients with ventilator associated lower respiratory tract infection (VA-LRTI). Use of appropriate treatment as early as possible would improve infection-related outcomes including increased risk of mortality and reduction in the spread of drug-resistant pathogens. Using samples collected from the international multi-centre BreathDx study [1], we aim to assess the diagnostic potential of measuring microbial volatiles of common respiratory pathogens in exhaled breath. We hypothesise that microbial volatiles in exhaled breath can be used to help diagnose pathogen-specific VA-LRTI.
Methods: Reference strains of four bacteria were cultured in nutrient broth (*S. aureus, P. aeruginosa, K. pneumoniae*, and *E. coli*) and their headspace volatiles sampled using an active and passive sampling method, as described previously [2]. Exhaled breath samples from ICU patients suspected of VA-LRTI were collected using Tenax GR packed sorbent tubes as previously described [1]. Samples were compared to parallel BAL culture data used as the reference for pathogen identification. Culture headspace and patient breath samples were analysed by thermal desorption-gas chromatography-mass spectrometry.
Results: 19 microbial VOCs were identified with high confidence from culture headspace of all bacteria. Production of discrete and shared microbial VOCs was observed across microbes and sampling methods. Breath samples were successfully collected from 45 patients with positive BAL culture, and 44 with negative BAL culture. Of those patients with positive culture, the following pathogens were identified relative to culture headspace experiments: *S. aureus* (n=15), *P. aeruginosa* (n=8), *E. coli* (n=2), *Klebsiella spp.* (n=5).
Conclusions: Core microbial VOCs were identified in headspace samples and will be used for constructing a semi-targeted method to search for these compounds in patient breath samples..

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Chad Schaber

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Monday 13, Auditorium, 16h10

Towards Standardization: Breath Biopsy® OMNI Assay for enhanced biomarker discovery

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Background: A lack of consistency in methodologies and reporting has limited progress in breath biomarker discovery and validation. In particular, failing to control for background and variability may have led to issues with false negative results.
Methods: At Owlstone Medical (OML), we have developed the OMNI platform, an end-to-end system optimized to provide reliable, reproducible results. The platform includes an improved breath collector, use of a high resolution OrbiTrap GCMS, robust quality control and standards, and tailor-made data-processing and analysis. A pilot study collected 57 exhaled breath samples from 4 volunteers at 4 days as well as 57 matched system blanks.
Results: The system blanks were used to determine a limit of detection (LOD) for each VOC, which is 3 standard deviations above the mean of the background. Only breath VOCs above the LOD are considered "on-breath". Even with these strict criteria, 392 compounds were found on-breath in at least 50% of breath samples.
Conclusions: Overall, the OMNI platform delivers significantly improved confidence for breath biomarker discovery on the road to breath-based diagnostic tests to save lives and healthcare costs. OMNI is already in use and has shown promising results in early customer studies. Finally, future work will continue to refine and improve capabilities including by providing a growing list of compounds measured by absolute quantitation rather than relative abundance.

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Tuesday 14 June

Session 7 Breath tests targeting unmet clinical needs

Chair: Jochen K. Schubert, Inger Lise Gade

Session 8 Data analysis and interpretation

Chair: Agnieszka Smolinska, Robert van Vorstenbosch

Session 9 Analytical methods for breath biomarker detection

Chair: Veronika Ruzsanyi, Bogusław Buszewski

Session 10 Breath analysis and cancer research

Chair: George Hanna, Daria Slefarska-Wolak

Session 11 Five shades of breath analysis

Chair: Jane Hill, Patricia Fuchs

Session 12 Volatile signatures of asthma in breath

Chair: Jane Hill, Patricia Fuchs

Tobias Walser

ETH Zürich, Zürich

Tuesday 14, Auditorium, 9h30

Zurich Exhalomics - Breath analysis at the forefront of research and clinical development

Tobias Walser (1), Steven A Brown (2), Imad El Haddad (5), Lukas Emmenegger (6), Philipp Gerber (3), Andreas Güntner (3), Malcolm Kohler (4), Alexander Möller (9), André Prévôt (5), Pablo Sinues (7), Emma Slack (1), Guy Vergères (8), Arnold von Eckardstein (4), Fabian Wahl (8), Renato Zenobi (1), Annelies Zinkernagel (4)

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Background: Bringing breath analysis closer to patients is the goal of the "Zurich Exhalomics" consortium founded in 2015 [1]. Fourteen research groups, from basic research to technology development and clinical studies, ensure that breath analysis is comprehensively advanced. The scale-up of the consortium required a new function: scientific coordination. This presentation provides insights into the strategy development and scientific coordination of this large-scale project. The presentation covers not only research activities, but also the approach to communication, public relations and fundraising.
Methods: The stakeholders of Zurich Exhalomics have a variety of needs. Understanding these and forming a coherent consortium rather than a loose network is one of the challenges in this multidisciplinary group. The coordinating body ensures that researchers can focus on their work, while the outside world understands and supports Zurich Exhalomics' activities ideally and financially. This continuous process requires a long-term vision and a strategy that can be easily adapted to a volatile environment. Speaking of volatiles, volatile molecules are at the heart of Zurich Exhalomics, with the aim of linking them to specific health conditions or diseases.
Results: The consortium's achievements include not only first-class publications, but also two spin-offs and the anchoring of the work in society through constant and strategic public involvement (media, events, foundations).
Conclusions: Zurich Exhalomics has evolved from an idea of a chemist and a medical doctor to a network of ambitious research groups in a short time. As time went by, a consortium has emerged with the common goal of bringing breath analysis closer to patients. Over the next five years, the project will focus on validating and quantifying biomarkers that are characteristic of diseases or therapies. In a further step, the library will then be expanded in a targeted manner to include further use cases. In parallel to these technologies, which are being developed towards marketability, basic research will be strengthened, especially in the areas of big data analysis, chronobiology, metabolomics and measurement technologies (sensors, mass spectrometry, laser spectroscopy).

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Camille Roquencourt Tuesday 14, Room 27, 9h30

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Processing and analysis of PTR-TOF mass spectrometry data for biomarker discovery in exhaled breath: application to COVID-19 intubated ventilated patient

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Background: The analysis of Volatile Organic Compound (VOCs) in exhaled breath is a promising non-invasive method for early diagnosis and therapeutic monitoring. Proton Transfer Reaction Time-Of-Flight Mass Spectrometry (PTR-TOF-MS) has recently emerged as an innovative technology for the real time analysis of exhaled VOCs [1]. However, there is currently a lack of methods and software tools for the processing of such breath data from cohorts [2,3].
Methods: We therefore developed a suite of algorithms that process raw data from several files and build the table of feature intensities in all samples, through 1) expiration and peak detection, 2) quantification, 3) alignment between samples 4) missing value imputation and 5) suggestion of feature annotation. Notably, we developed innovative 2D peak deconvolution method based on penalized splines signal regression, allowing to select VOCs which are specific from exhaled breath, and discarding environmental contamination.
Results: We implemented all methods in a new software tool, publicly available as the ptairMS R package on Bioconductor, as well as a graphical interface [4]. The approach was validated on both simulated and experimental datasets, and we showed that the precision and recall of the VOC detection reached 99.99% and 98.4%, respectively, and that the error of quantification was below 8.1% for concentrations down to 19 ppb. Finally, we applied our methodology to the clinical characterization of exhaled breath from 40 mechanically ventilated adults with COVID-19 Acute Respiratory Distress Syndrome. We are able to diagnosis the infection with an accuracy of 93% using a 10-fold cross validation repeated four times, and we identified four biomarkers, thanks to a longitudinal follow-up depending on hospitalization time using a mixed model [5].
Conclusions: We developed new software tools and methods for real time analysis of PTR-TOF-MS data from exhaled breath for biomarker discovery, and permit to identified four biomarkers of COVID-19 infection in exhaled breath of ARDS ventilated patient.

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Fabio Di Francesco Tuesday 14, Auditorium, 9h50

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Breath biomarkers of heart failure

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Background: Heart failure (HF) is a complex syndrome caused by structural and/or functional abnormalities of the heart and a main cause of mortality and poor quality of life in western societies. Patients are periodically hospitalized due to the exacerbation of symptoms, and prevention of acute conditions would slow down the disease progression. The painless and non-invasive breath analysis is attractive to support conventional clinical investigations, for example by a point of care device to be used from patients for self-monitoring at home. We present results of studies aimed at verifying the possibility to use breath VOCs to monitor HF patients' conditions.
Methods: Different NTDs, i.e. triple-beds, packed with 1 cm of each Divinylbenzene, Carbopack X and Carboxen 1000, or single packed with 3 cm Tenax GR 60/80 mesh, were used to analyze VOCs (i.e. hydrocarbons, ketones, aldehydes, aromatics and sulfurs, [1]) in aliquots (50 mL) of mixed breath samples, which were collected from heart failure patients. Forty-five hospitalized heart failure patients were monitored at hospital admission and at hospital discharge, and a second group of chronic HF patients (n=25) were monitored before and during a cardiopulmonary stress test exercise. In the second group, SIFT-MS was used to monitor in real time breath concentrations of acetone and isoprene.
Results: About 80% of the patients monitored at the hospital showed significantly higher breath acetone levels (a factor of 3 at least) at admission (acute phase) compared to discharge. Similar results were found during the exercise tests, during which significantly higher breath acetone concentrations were found at peak effort condition compared to rest condition. A strong correlation between breath acetone and plasma levels of NT-type brain natriuretic peptide was observed.
Conclusions: The analytical procedure for the determination of VOCs in exhaled breath by NTME followed by GC-MS/MS analysis proved to be very reliable. Breath acetone seems to be the most promising candidate biomarker to monitor health conditions of HF patients.

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Robert van Vorstenbosch Tuesday 14, Room 27, 9h50

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The detection of primary sclerosing cholangitis using an optimized methodology for fecal VOC analysis using the microchamber thermal extractor

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Background: In Primary Sclerosing Cholangitis (PSC) inflammation of the bile ducts results in accumulation of waste products in the liver, causing liver damage and cirrhosis. Approximately 8% of Irritable Bowel Disease (IBD) patients will develop PSC, highlighting a need for non-invasive screening tests. Recently, exhaled breath could successfully distinguish IBD from PSC. In the current study, we show similar utility of fecal VOC profiles.
Methods: Fecal samples of 24 PSC patients and 49 IBD patients were sampled using optimized settings of the Micro-Chamber/Thermal Extractor (MC). Next, fecal VOC profiles were analyzed using Gas Chromatography coupled to Mass spectrometry (GC-MS). Due to high variation in the data, data normalization was unfeasible. Therefore, a log-ratio approach combined with Random Forest was applied to analyze the data [1]. To improve predictions, data fusion of exhaled breath and fecal VOC profiles was performed using proximity stacking [2].
Results: A semi-targeted approach to distuinguish PSC from IBD cases resulted in an AUC ROC of 0.82 based on 8 fecal VOC markers. Proximity stacking further increased these predictions.
Conclusions: Fecal VOC profiles were shown to distinguish PSC from IBD and offer complementary information compared to exhaled breath profiles, thereby offering unique insights into the microbiome-gut-liver axis related to PSC.

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Renate Kos Tuesday 14, Auditorium, 10h10

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Targeted exhaled breath analysis for detection of respiratory pathogens in cystic fibrosis patients

Renate Kos (1), A. H. Neerincx (1), P. Brinkman (1), T. Paff (2), M. G. Gerritsen (1), D. W. Fenn (1)(3), J. C. Davies (4), C. J. Majoor (1), E. G. Haarman (2), A. H. Maitland-Van Der Zee (1,2), on behalf of the Amsterdam Mucociliary Clearance Disease (AMCD) Research Group and the Amsterdam UMC Breath Research Group.

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Background: Recurrent respiratory infections are associated with worsening lung function in patients with cystic fibrosis (CF). Culture dependent detection of such infections is time-consuming and often relies upon sputum. Volatile organic compounds (VOCs) in exhaled breath may allow for rapid non-invasive pathogen identification. We adopted a targeted approach and aimed to 1) identify VOCs of interest in literature and 2) assess the diagnostic accuracy of exhaled breath analysis to detect common CF pathogens: *Staphylococcus aureus* (SA), *Aspergillus fumigatus* (AF), and *Haemophilus influenzae* (HI).
Methods: VOCs for each pathogen were firstly identified through a literature review and selected based on multiple reporting's. Data from an independent cross-sectional study of CF patients was used to evaluate their validity. Pathogen presence was defined as a positive culture at visit/chronically infected. VOCs were identified using a quadrupole MS. The primary endpoint was the area under the receiver operating characteristics curve (AUROCC) of individual VOCs and VOCs combined in a multivariable regression model, for each pathogen.
Results: Over 400 VOCs were identified in literature of which 65 were selected for further analysis. 8 SA related, 1 AF related, and 8 HI related VOCs could be detected in the exhaled breath of 25 paediatric and 28 adult CF patients. Individual VOCs related to SA (positive=31, free=11), and HI (positive=9, free=28) had an AUROCC of 0.47-0.74 and 0.46-0.62, respectively. For AF (positive=6, free=36), the identified VOC had an AUROCC of 0.47 (CI: 0.23-0.72). Combined VOC analysis showed improved discrimination with AUROCC of 0.78 (CI: 0.62-0.93) for SA and AUROCC of 0.82 (CI: 0.66-0.98) for HI.
Conclusions: Targeted VOC analysis showed promising results for the detection of pathogens, and that composite VOC fingerprints were superior to individual VOCs. This study shows the potential of hypothesis driven breath analysis for pathogen detection.

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Celia Mallafré-Muro Tuesday 14, Room 27, 10h10

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Breath analysis for the detection of pseudomonas aeruginosa infections in bronchiectasis patients using electronic nose and gas chromatography-mass spectrometry

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Background: Bronchiectasis is a bronchial pathology that can present exacerbation episodes caused by some opportunistic bacteria. In this case, breath samples from patients with stable bronchiectasis and, from patients with exacerbation episodes caused by Pseudomonas Aeruginosa (PA), were collected and analyzed with a GC-MS and an E-Nose.
Methods: The breath of 13 bronchiectasis patients, 12 bronchiectasis patients suffering a PA infection, and 9 controls was collected into Tedlar Bags [1]. These samples were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and electronic nose (e-Nose). The e-Nose was a commercial Cyranose 320 with an array of 32 nanocomposite sensors. The data obtained were analyzed with the aim of discriminating the health condition. Chemometric methods were applied, and the obtained results were evaluated with blind samples for the data obtained from the GC-MS.
The data obtained from the e-Nose, for improving the gaussianity, was transformed using a non-linear arctangent transformation [2]. K-NN models were built and the classification rates obtained were tested with a double leave one subject out (LOSO) cross-validation [3,4].
Results: The e-Nose breath analysis was able to separate the 3 groups with a K-NN classification rate of 84% in the 3 classes classification problem. When comparing 2 by 2 classes, the classification rates varied between 84 to 100%, obtaining perfect discrimination between control and bronchiectasis with PA infection samples. These results were tested with external double cross-validation and confirmed by a permutation test. Regarding the GC-MS analysis, the discriminant analysis using PLS-DA reported good results that were not statistically significant in the permutation test.
Conclusions: The breath analysis carried with the e-Nose, followed by a strict and proper data analysis, is able to discriminate successfully between control and patients with bronchiectasis and exacerbation episodes. Additionally, GC-MS needs further experiments to increase the number of patients so statistically significant results can be reached.

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Tuesday 14, Auditorium, 10h30

Idealised lung clearance indices for paediatric patients

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Background: The lung clearance index (LCI) assesses lung function by quantifying the number of 'lung turnovers' required to reduce the exhaled concentration of a tracer gas to 1/40th of its starting value. The LCI is often assumed to be a proxy for ventilation inhomogeneity and has been shown to be a sensitive method of measuring lung abnormalities in adult and paediatric cystic fibrosis (CF) patients[1]. There are limitations with this measure, however, associated with a lack of standardisation and specification of equipment and procedure[2,3]. To address these limitations, a new lung function assessment has been developed, providing an idealised-LCI (iLCI).
Methods: A molecular flow sensor (MFS) records highly-accurate measurements of respiratory gas flux at the mouth during multiple breath washout experiments[4]. The highly-precise data gathered by this sensor, coupled with the development of a lung model, allows estimation of clinically significant parameters describing ventilation and perfusion inhomogeneity[5,6]. The iLCI is generated by performing a series of simulated multiple breath washout experiments from a person-specific modelled lung. By 'turning off' various aspects of lung inhomogeneity within the simulations, the physiological origins of the iLCI can be quantified.
This method has been used to assess adult patients with CF and a control group. The additional volume associated with this adult MFS renders it inappropriate for use with paediatric patients. To this end, a paediatric MFS has been developed in which the volume has been reduced from 50 to 12 ml.
Results and Conclusions: The paediatric MFS has a precision comparable to the adult device, enabling iLCIs of paediatric patients to be assessed for the first time. The iLCI correlates well with LCIs measured with standard methods but shows better separation between CF patients and healthy control group. In addition, the iLCI provides a breakdown of the physiological origins of the iLCI values, demonstrating that abnormalities are not exclusively driven by ventilation inhomogeneity.

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Monika Śmiełowska Tuesday 14, Room 27, 10h30

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Screening for volatile biomarkers of colorectal cancer by analyzing breath and fecal samples using thermal desorption combined with GC-MS (TD-GC-MS)

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Background: Volatile organic compounds (VOCs) are gaining increasing use in disease diagnosis. Compared to classical methods, they are characterized by high simplicity and non-invasiveness at the sampling stage [1]. However, a key challenge is to provide analytical tools that ensure the ability to identify volatile biomarkers of a disease. The purpose of this presentation is to demonstrate the results of a study on the identification of VOCs present in exhaled air and feces of healthy volunteers and colorectal cancer patients. An important part of the research was chemometric analysis aimed at preliminary identification of potential volatile biomarkers of tumor disease.
Methods: Tedlar bags were used for the air sampling. For collection of gas phase released from feces, emission microchambers were applied. Sorption tubes were used to enrich analytes for both breath and fecal samples. Thermal desorption technique combined with gas chromatography coupled with mass spectrometry was used at the separation and identification step [2].
Results: Identification of VOCs, based on comparison of the obtained results with a standard library of mass spectra, made it possible to determine the presence of numerous chemicals in breath and fecal samples. The obtained profiles allowed the samples to be differentiated according to the patient's health status.
Conclusions: The study is the first attempt to document the potential of using TDGC-MS to analyze both breath and fecal samples to search for volatile biomarkers of colorectal cancer. A full evaluation of the results described herein requires further studies involving a larger number of samples and analysis of volatile fraction released from cancer tissues. Moreover, it is particularly important to understand the metabolic pathways of substances postulated as tumor markers.
Acknowledgements: This work was supported by The National Centre for Research and Development (Warsaw, Poland) "Airborne Biomarkers for Colorectal Cancer" project (ERA-NET TRANSCAN/023/2018).

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Bogusław Buszewski Tuesday 14, Auditorium, 11h20

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Comparative study of breath and fecal samples

Bogusław Buszewski (1)(2), Tomasz.Ligor (1,2), Monika Śmiełowska (2), Tomasz Kowalkowski (1)(2), Marek Jackowski (3), Wojciech Kupczyk (3), Jacek Szeliga (3), Frederik-Jan van Schooten (4), Agnieszka Smolińska (4), Jonathan Beauchamp (5)

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Background: Volatile organic compounds (VOCs) identified in exhaled breath and fecal samples could provide information regarding biochemical processes in the human body. Morever, such methodology can allow for and non-invasive screening procedures to diagnose and monitor colon diseases. We have to focus on searching for characteristic volatiles in the breath and in the fecal samples of the patients to provide set of potential biomarkers of colon cancer [1].

Methods: Micro‑chamber thermal extractor (μ‑CTE) was applied for extraction and preconcentration volatiles from fecal samples. Samples were preconcentrated on Tenax TA/Carbograph 5TD Bio Monitoring tubes followed by thermal desorption (TD). Gas chromatography coupled with mass spectrometry (GC/MS) was used for identification and quantitation of VOC. Results were evaluated by means of chemometric methods [2].

Results: Application of developed methodologies to VOC analysis in breath and fecal samples allows to identify hundreds of compounds. Considerable variation of VOC between samples were observed. Volatile fatty acid, especially short chain molecules as well as phenols and indol were mainly found in stool. Unlike breath samples, acetone and isoprene were not identified.

Conclusions: Two different matrices, such as breath and stool samples were evaluated. Samples show considerable variation in the composition of the VOC in breath and fecal. The same chromatographic conditions has been applied. Statistical tools for data processing provided model to compare breath's and fecal's volatiles. Specific “VOC- print” show fine differences between patients and control. Some potential biomarkers were identified.

Acknowledgements: This work was supported by The National Centre for Research and Development (Warsaw, Poland) "Airborne Biomarkers for Colorectal Cancer" project (ERA-NET TRANSCAN/023/2018).

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Tuesday 14, Room 27, 11h20

Breath analysis for early detection of pulmonary pathologies as malignant pleural mesothelioma

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Background: The development of non-invasive diagnostic approach able to identify patients at risk of developing cancer and asymptomatic with early stage cancer, is the key-challenge of the scientific community. The identification of a pathology-related Volatile Organic Compounds (VOCs) pattern in the human breath is recognized as promising methodological approach for the early diagnosis of oncologic diseases as malignant pleural mesothelioma (MPM) [1]. MPM is a rare neoplasm mainly caused by asbestos exposure with a high mortality rate. The management of patients is controversial due to a long latency period of MPM and non-specific symptoms appearing at advanced stage of the disease [2].
Methods: In this study a methodological approach able to identify a VOCs pattern allowing a discrimination between healthy and sick clinical conditions was developed and validated. Breath samples collected from patient affected by MPM, healthy controls (CTRL) and symptomatic asbestos-exposed persons (AEx) were directly collected on sorbent tubes (biomonitoring, Markes) by an automatic breath sampler Mistral (Predict srl) and analysed by TD-GC/MS (TD Markes Unity 2 - GC Agilent 7890/MS Agilent 5975) [3].
Results: Nonparametric test as Wilcoxon/Kruskal Wallis tests (R version 3.5.1) allowed to identify the most weighting variables to discriminate between MPM, CTRL and AEx breath samples. On the basis of p-values lower than 0.05 and current knowledge on metabolic processes, a multivariate statistical approach was applied at collected data considering only selected variables.
Conclusions: Considering that MPM is an aggressive neoplasm leading to a late diagnosis, a promising data mining approach was developed and validated in order to discriminate between MPM, CTRL and AEx. Leave-one-out cross-validation method was applied to calculate the prediction accuracy, sensitivity and accuracy and specificity

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Wolfgang Vautz

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Tuesday 14, Auditorium, 11h40

From security to health: Breath-based information obtained by GC-Ion mobility spectrometry

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Background: Ion mobility spectrometry (IMS) is a well-known method for trace gas detection for civil security and military purposes. Recently, coupling IMS with fast gas-chromatographic (GC) pre-separation enabled sensitive and selective analysis of complex mixtures.
Methods: GC-IMS opened up new fields of application such as breath analysis. While mobile standalone IMS systems were already available, the use of MEMS-based in-line pre-concentration along with µGC brought further progress including enhanced selectivity and immediate on-site results.
Results: Implementing mobile GC-IMS enabled successful development of innovative applications:

- On site detection of drug abuse by analysis of a single exhaled breath [1].
- Detection of hidden persons after only 30 min in a container or lorry trailer by analysing the air for "signs of life" [2].
- Quantification of levels of medicinal drugs in breath was demonstrated successfully for anaesthetics during surgery from human breath [3] and even for mice [4].
- Cultivated bacteria could be identified after few hours (3-6 h) from the patterns of metabolites they emanate [5]. There are also indications that resistant and non-resistant variations could be differentiated. The next step is the identification of bacteria directly from exhaled breath, thus avoiding time-consuming cultivation and allowing earlier specific, lifesaving anti-biotic therapy.
- Not only infections but also diseases change the human metabolism and thereby, the composition of exhaled metabolites, as successfully demonstrated in nephrology [6].

Conclusions: The above-mentioned applications demonstrate the potential of IMS coupled to fast GC pre-separation for the analysis of - not only - human breath. A very current topic is the rapid, proofed on-site detection of viral infections. Several studies have already demonstrated the potential of allowing this, but further research is required, which will be one of the top issues for research in the near future.

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Sarah Haywood-Small Tuesday 14, Room 27, 11h40

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Volatile organic compound analysis of a chorioallantoic membrane model within malignant pleural mesothelioma

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Background: Malignant pleural mesothelioma (MPM) represents a significant diagnostic challenge; typically detected at an advanced stage and precluding curative treatment. Novel and non-invasive biomarkers may allow earlier detection and improve patient management and outcomes [1]. Previously, we reported that different histological MPM sub-types produce distinct volatile organic compounds (VOC) profiles [2]. Our new study extends the VOC analysis methods towards more complex and vascularised 3D tumour structures, using a chick embryo chorioallantoic membrane (CAM) model (MPM-CAM) [3].

Methods: MPM cells from various histological sub-types and genetic backgrounds (MESO-7T, MESO-8T and MESO-12T from MesoBank, UK) were grown on the CAM as vascularised xenografts. Tumours were imaged to confirm viability, dissected, and stored in RNAlater. VOCs were extracted using a published method for lung carcinoma samples [4]. Sealed headspace vials were incubated at 37°C for 60 minutes with continuous solid-phase microextraction (SPME). Significantly altered variables were imported into SIMCA (V15.0.2; Umetrics) for multivariate statistical analysis. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to visualise similarities and differences between the groups.

Results: Background deduction was performed through t-tests. PCA and PLS-DA analysis of CAM-specific VOCs showed separation between the various groups. Across all sample groups, six compounds were identified as significantly different VOCs. Isopropanol and diethylene glycol dipivalate showed a higher relative intensity in the biphasic (MESO-7T) compared to the epithelioid (MESO-8T and MESO-12T) groups.

Conclusions: Data confirms the VOC profile of various MPM sub-types using a unique CAM-MPM model. Data may be clinically relevant in the pursuit of developing a breath test for MPM. These specific VOCs could act as non-invasive biomarkers, enabling early diagnosis of MPM.

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Thomas Wortelman Tuesday 14, Auditorium, 12h00

G.A.S. Dortmund mbH, Dortmund NRW Department of Internal Medicine, Rotes Kreuz Krankenhaus, Bremen, Germany

GC-IMS plus various sampling Techniques to test for individual Volatiles at sup-ppb level

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Sampling: The GC-IMS is designed to give a wide choice of how the analyst wants to sample. With the high sensitivity of the IMS detector, molecules in the sub-ppm concentration range can be detected immediately in many applications. However, in order to be able to measure quantitatively and reproducibly, the greatest care must be taken. Various applications such as measurements of skin emissions or from gas bags have been developed, the time-consuming calibration for quantification can already be automated to a large extent.

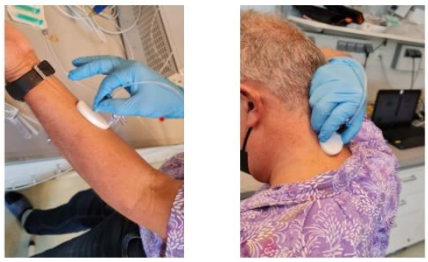

Fig. 1. Sampling to test skin volatiles (G.A.S.).

Advantages of IMS as a complementary detector coupled to high-end GC-MS: The analyst can perform sample preparation and molecular separation with the benchtop GC system and also record ion mobility spectra in parallel with a mass selective detector (MSD) allowing the compilation/extension of a customized breath related library.

Fig. 2. Agilent 6890N GC with 5973 MSD (Agilent) and IMS (G.A.S.)

µ-Thermal Desorber (µTD)-GC-IMS coupling: Active aspiration of the sample, collection and pre-concentration with automated sample application allows 24/7 monitoring in various applications with detection of volatiles in the sub-ppm range.

Fig. 3. Coupling of µ-TD (AIRSENSE) with GC-IMS plus N2-Generator (G.A.S.)

Short 'cycle times' are interesting for continuous monitoring via by-pass or for respiratory gas analysis when using gas bags. The operating gas supply unit allows trace gas analysis in an industrial environment or for mobile use on site.

GC-IMS with Lab-Thermal Desorber: To measure respiratory gas, the BreathSpec® was successfully coupled with a thermal desorber for discontinuous measurement of respiratory gas samples trapped on tubes.

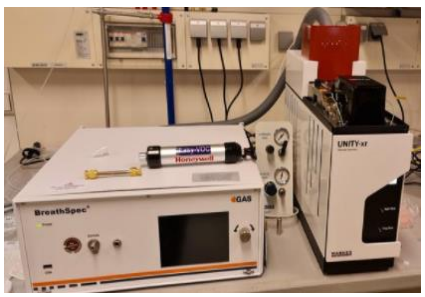

Fig. 4. BreathSpec® (G.A.S.) coupled to a Thermal Desorber (MARKES Unity-xr).

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Eline Schillebeeckx Tuesday 14, Room 27, 12h00

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Breath analysis allows to predict treatment response in malignant pleural mesothelioma patients

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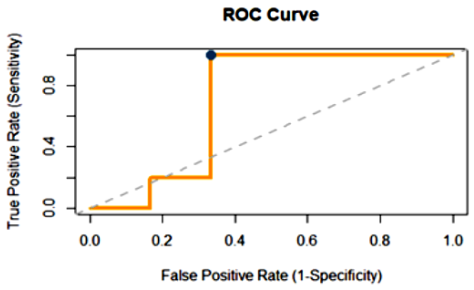
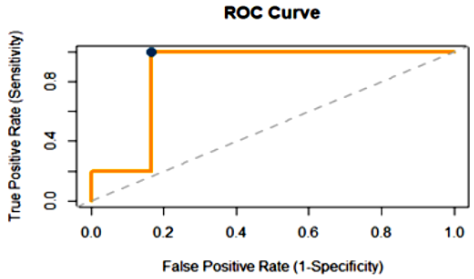
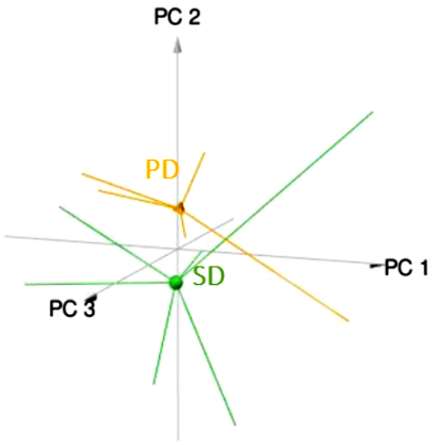
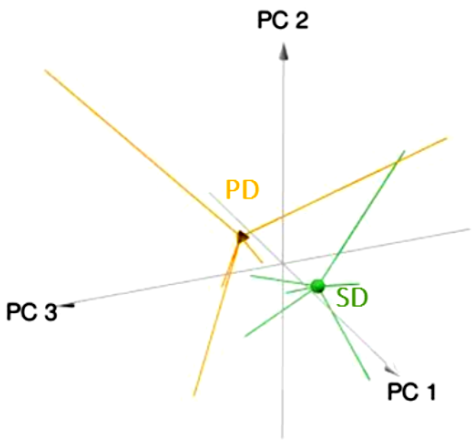
Background: The current follow-up procedure for malignant pleural mesothelioma (MPM) patients is not optimal and predictive markers are lacking. Our aim is to investigate whether volatile organic compounds (VOCs) in exhaled breath are able to differentiate patients with different treatment outcome in follow-up samples and, if so, if this outcome can be predicted earlier on.
Methods: At least one breath and background sample was collected from 9 MPM patients via multi-capillary column-ion mobility spectrometry. The associated CT-scans were scored as either stable (SD) or progressive (PD). After background correction, a lasso regression was performed. A discriminative model was created to differentiate between SD and PD in follow-up samples, while a predictive model was trained to predict the follow-up outcome based on the associated breath sample from the previous study visit.
Results: We were able to differentiate between SD and PD in follow-up samples with an accuracy of 81.8% (95% CI 51.8-96.8). Furthermore, a baseline breath sample was able to predict treatment outcome with an even higher accuracy (table 1). However, due to the small samples size, it is to be expected that our confidence intervals are relatively large, which is why validation in a larger population is necessary.
Conclusions: VOCs in exhaled breath are promising in detecting and predicting treatment outcome of MPM patients. However, further research in a larger population is necessary to validate our results.

Table 1. An overvew of the variables, their distribution, the selected peaks and receiver operating characteristic for both models. The principle component analysis plots visualize the discrimination between samples linked to SD an PD (discriminative model) and the discrimination between baseline samples based on treatment outcome (predictive model).
AUC = area under the receiver operating characterisric curve, BMI = body mass index, carbo/ralti = carboplatin raltitrexed, cis/pem = cisplatin + pemetrexed, F = female, M = male, NPV = negative predictive value, PCA = principle component analysis, pem mono = pemetrexed mono therapy, PPV = positive predictive value, PD =

progressive disease, ROC = receiver operating characteristics, SD = stable disease, TB = treatment break, TN = treatment naïve.
* inclusion and follow-up samples. Treatment naïve or under treatment.
** mean age and BMI with standard deviations.

	DISCRIMINATIVE MODEL			PREDICTIVE MODEL		
	SD	PD	p-value	SD	PD	p-value
N*	*6*	*5*	*/*	*6*	*5*	*/*
AGE (years)**	*67,83 (±7,2)*	*69,41 (±6,4)*	*0,708*	*66,97 (±7,3)*	*68.97 (±6,4)*	*0,640*
BMI (kg/m^2)**	*23,48 (±3,9)*	*22,21 (±2,1)*	*0,513*	*22,92 (±3,9)*	*23.85 (±1,8)*	*0,617*
GENDER (% male/% female)	*50/50*	*80/20*	*0,546*	*50/50*	*80/20*	*0,546*
SMOKING STATUS (% non/% current/% ex)	*67/0/33*	*30/0/60*	*0,567*	*67/0/33*	*30/0/60*	*0,567*
TREATMENT (%)	*50 Cis/pem 16,6 Nivolumab 16,6 Pem mono 16,6 Dendritic cell vaccine*	*40 Nivolumab 20 TB, 20 Cis/pem 20 Gemcitabine/ Ramucirumab*	*0,482*	*33,3 Cis/pem 33,3 TN 16,7 Carbo/ralti 16,7 Pem mono*	*60 Cis/pem 20 Nivolumab 20 TN*	*0,382*
SELECTED VOCs	*P10, P21, P32, P43, P54, P65, P76, P87*			*P10, P21, P32, P43, P54, P65, P76, P87, P4, P15, P26, P37, P48, P59, P70, P81, P92*		
AUC (%)	*70,0 (33,3-100)*			*86,7 (60,0-100)*		
SENSITIVITY (%)	*100 (54,9-100)*			*100 (54,9-100)*		
SPECIFICITY (%)	*66,7 (26,2-94,0)*			*83,3 (40,9-99,2)*		
PPV (%)	*71,4 (33,1-94,9)*			*83,3 (40,9-99,2)*		
NPV (%)	*100 (47,3-100)*			*100 (54,9-100)*		
ACCURACY (%)	*81,8 (51,8-96,8)*			*90,9 (62,7-99,5)*		
ROC curve						
PCA plot						

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Antonello Laricchiuta Tuesday 14, Auditorium, 12h20

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From security to health: Breath-based information obtained by GC-Ion mobility spectrometry

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Two new breath collection and sample preparation techniques are presented that significantly improve sensitivity and range of recoverable compounds while reducing the level of matrix interferences found with other approaches. These new solutions provide a means to perform GC/MS, GC/TOF, GC/MSMS, GC-Orbitrap, and GCxGC methods on enriched extracts without the common interferences created by water vapor, CO_2, and typical non-volatiles found in aerosols within the breath (proteins, lipids, carbohydrates, bacteria/viruses, inorganics, etc). Elimination of these matrix interferences is critical to establish reliable solutions for accurate breath measurements. Two techniques are presented; one that collects breath condensate using a simple oral rinse followed by vacuum extraction within a vial, and the second approach using a glass vacuum sampling container with a sorbent device present within the sampling device. In the first approach, a technique called VASE – Vacuum Assisted Sorbent Extraction, is performed by attaching a sorbent to the top of a vial containing a liquid or solid sample, and a microseal in the sorbent device (Sorbent Pen) allows a vacuum to be created on the sample. For an oral rinse using water or an NaCl saline solution, a vacuum down to about 1/30th of an atmosphere can be generated at 25 deg C on the vial/sorbent, after which the vacuum source is removed, leaving a headspace extraction process that proceeds much faster than would occur at atmospheric pressure. During vacuum extraction, the combined vial/sorbent "closed system" can be elevated in temperature to increase the vapor pressure and therefore transfer rate of heavier or highly polar organic compounds to the sorbent. Dehydration of the sorbent can occur after sample extraction by cooling the bottom of the vial below the temperature of the sorbent. In the second approach a special adaptation of the VASE approach results in a sampling device that places the sorbent device inside of a 0.5-1L glass container that is cleaned and evacuated prior to collecting a gas phase breath sample. Unlike a Tedlar bag where compounds can adsorb onto the walls and are thus not recovered when analyzing the gas phase content in the bag, compounds in breath that condense onto the walls of a glass bottle are in equilibrium between the walls and the gas phase, and will eventually transfer to the sorbent. Recovery of lower vapor pressure breath compounds can be accelerated by drawing a vacuum on the vial through the sorbent, and then heating the bottle and sorbent to complete the transfer of heavier compounds.

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Kiran Sankar Maiti Tuesday 14, Room 27, 12h20

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Diagnosis of prostate cancer via infrared spectroscopy of breath

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Background: Prostate cancer (PC) is the most common cancer of men in Europe, North America, and some parts of Africa [1]. In the death ranks, PC is the third among all cancerrelated death of men. The high mortality rate of the PC is due to the lack of adequate diagnostics in the early stage. The existing non-invasive diagnostics of PC suffer from low accuracy (<70%) even at advanced stages.
Methods: We are developing a breath analysis-based non-invasive diagnostic method using infrared spectroscopy [2]. The identification and quantification of the metabolites present in exhaled breath is the key of this diagnostic method [3]. The advantage of infrared spectroscopy is based on the fact that all biological molecules have unique absorption spectra (fingerprints) in the spectral range 500 - 4000 cm^{-1}. The basic idea of this diagnostic method is to investigate changes in the metabolic composition of human breath at the onset of the disease.
Results: For a demonstration, we have studied the exhaled breath of a small group of PC patients and compared them with healthy volunteers [4]
. Kidney cancer (KC) and bladder cancer (BC) which occur at the same physiological system as PC are chosen to explore the ability of the technique to distinguish among three cancer groups. We identified eight specific spectral regions to distinguish between cancer and healthy cohorts. With unsupervised and supervised statistical analysis, a clear separation of healthy and cancer cohorts was observed. For five spectral regions, the accuracy of identification of two cohorts exceeded 95%.
Conclusions: Infrared spectroscopy is already proved its ability to identify and quantify the metabolites in gaseous biological samples. A pilot study with prostate cancer cases shows a promising result to distinguish cancer cases from healthy volunteers. The separation between PC, BC, and KC are not prominent, maybe due to the smaller sample size. An investigation with a larger sample size is already planned.

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Theo Issitt

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Tuesday 14, Room 27, 12h40

Identification of *Burkholderia pseudomallei* infection using patient breath

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Background: Diagnosis of cancer has been shown to be possible using volatile organic compounds (VOCs) present in the breath [1]. VOCs of interest can be found in breath but these potential biomarkers may be hidden in background 'noise', leading to reproducibility challenges in breath diagnostics. Translational biomarker research, where we consider which aspects of the disease translates to human breath, such as tumour hypoxia, may offer a more promising approach for the discovery of diagnostic targets.

Methods: Using gas chromatography mass spectrometry and a multi-time point sampling method to investigate VOC metabolisms of environmental VOCs, we explore the outputs arising from cellular environmental conditions associated with cancer pathologies, such as hypoxia and glucose starvation. This approach was then applied to a xenograft mice models of breast cancer.

Results: Using 12 select VOCs (including methyl halides), linked by metabolic process, clear treatment specific responses were observed (VOC 1 ****p = 0.0001; VOC 3, 8, 12 ***p = 0.001; VOC 6, 9, 11 *p = 0.05). Principal component analysis revealed clear separation of groups with 62.8% explained variance for hypoxic conditions and 57.3% for glucose starvation. These results translated to our mouse model, and through targeted and non-targeted analyses, we have identified diagnostic compounds which we can target in human breath.

Conclusions: Targeting cellular stress from starvation or hypoxia to model pathophysiological environments could be useful in identification of VOC biomarkers. We have shown that longitudinal approaches to investigate active VOC metabolisms by cells and mice can separate disease models from control. These approaches can be applied to a variety of diseases for further translation biomarker discovery.

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Inger Lise Gade Tuesday 14, Auditorium, 14h30

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Bottom-up proteomic analysis of the exhaled breath condensate from twenty-six individual healthy persons

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Background: Exhaled breath condensate (EBC) is a promising source for detection of new biomarkers of diseases [1]. Investigation of the normal protein content in EBC is a prerequisite for future search for biomarkers. We have investigated the protein composition of individual EBC samples from 26 healthy persons.

Methods: We collected and analyzed EBC samples from healthy subjects recruited among out-patients at the Department of Cardiology at Aalborg University Hospital. Subjects were followed for common, benign cardiologic conditions such as atrial fibrillation. The EBC samples were stored at -80°C until analysis. The protein composition of the 26 individual EBCs were analyzed by nano liquid chromatography–tandem mass spectrometry. MaxQuant and subsequently Perseus was used for label-free quantification analysis, data management and differential expression analysis. Proteins classified as potential contaminant, identified only by site or in the reverse database, or by only one unique peptide were excluded from further analysis. Bioinformatic analysis was based on proteins present in at least 70% of the samples.

Results: The mean collected EBC volume was 4.1 mL/30 minutes (range 1.8 – 7.0 mL). A total of 534 proteins were identified with at least 2 unique peptides in the 26 EBC samples, highest number of unique peptides was 37 (Apolipoprotein B-100). The average number of proteins identified in the EBC samples were 141 (range: 53 – 433), 15 proteins were identified in all 26 EBC samples. Fourty-two proteins were present in at least 70% of the samples. The majority of these proteins are secreted from cells into the interstitial fluid or blood (i.e. extracellular space), blue nodes in Figure 1. Many of these proteins were involved in immune system processes (Red nodes in Figure 1).

Conclusions: The human EBC contains a variety of endogenous proteins, but only a minor fraction of the proteins were identified in all 26 EBC samples in our study. Actively secreted proteins are represented in the EBC, many of which have functions related to immune system processes in helathy individuals.The EBC may be a source for new diagnostic markers of diseases, but refinement of single sample analysis is needed for description of the proteome of EBC.

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Mahmoud I. Abdel-Aziz Ibrahim Tuesday 14, Room 27, 14h30

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Exhaled VOCs are linked to house dust mite-atopy in asthmatics and wheezers: results from the U-BIOPRED cohorts

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Background: Exhaled breath volatile organic compounds (VOCs) eNose profiles have been associated with atopic asthma across different age groups [1]. House dust mite (HDM) is one of the most common aeroallergens that plays a role in asthma pathophysiology. In this study, we aim to identify the VOCs that are differentially abundant in HDM-atopic and non-atopic pediatric and adult subjects with asthma or wheezing.
Methods: Exhaled breath samples were collected from U-BIOPRED pediatric and adult patient cohorts and samples were processed for gas chromatography-mass spectrometry (GC-MS) as previously described [2]. HDM atopy was defined as; a positive skin prick test ($\geqslant$3mm) and/or a positive specific IgE ($\geqslant 0.35 kU \cdot L^{-1}$) to Dermatophagoides pteronyssinus and/or farinae. Pediatric U-BIOPRED was used as a discovery cohort, in which differentially abundant ion fragments (DAFs) between HDM-atopic and non-atopic groups with nominally significant p-values (<0.05), after univariate Mann-Whitney U test,

were selected. Adult U-BIOPRED was used as a validation cohort, in which only matching DAFs were selected for putative compound identification and multivariate Partial Least Square-Discriminant Analysis (PLS-DA) with reporting areas under receiver-operating characteristic curves (AUROCCs) (95% CI).
Results: A total of 102 pediatric asthmatic and wheezing children (mean age: 7.4±4.6 yrs, 37.3% females, 45.1% school-aged asthmatics, and 44.1% HDM-atopic) and 52 adult asthmatics and healthy controls (mean age: 48.3±15.4 yrs, 53.8% females, 90.4% asthmatics, and 48.1% HDM-atopic) were included in the discovery and validation cohorts, respectively. Five replicated DAFs between the HDM-atopic and non-atopic groups were found and putatively identified as methacrolein, 1-methylthio-1-propene, 2-ethyl-1-hexanol, and one remained unidentified. These DAFs exhibited AUROCCs = 0.78 (95% CI 0.68-0.88) and 0.85 (95% CI 0.74-0.96) in the discovery and validation cohorts, respectively.
Conclusion: Breath analysis revealed distinct VOC signatures associated with HDM-atopy in children and adults with asthma or wheezing. These findings suggest that exhaled breath VOCs may be used in asthma phenotyping.

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Pritam Sukul Tuesday 14, Auditorium, 14h50

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Effects of COVID-19 protective face-masks and wearing durations onto respiratory-haemodynamic physiology and exhaled breath constituents

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Background: While assumed to protect against coronavirus transmission, face-masks may have effects on respiratory-haemodynamic parameters. Here, we investigated immediate and progressive effects of FFP2 and surgical masks on exhaled breath constituents and physiological attributes in 30 adults at rest [1].

Methods: We continuously monitored exhaled breath profiles within mask space in older (age: 60–80 years) and young to mid-aged (age: 20–60 years) adults over the period of 15 and 30 min, respectively by high-resolution real-time mass-spectrometry (PTR-ToF-MS). Peripheral oxygen saturation, respiratory- and haemodynamic parameters were measured (noninvasively) simultaneously.

Results: Profound, consistent and significant (p-value≤0.001) changes in SpO2 (Adults>60_FFP2-15min: 5.8±1.3%$^{-}$, Adults>60_surgical-15min: 3.6±0.9%$^{-}$, Adults<60_FFP2-30min: 1.9±1.0%$^{-}$, Adults<60_surgical-30min: 0.9±0.6%$^{-}$) and pET-CO_2 (Adults>60_FFP2-15min: 19.1±8.0%, Adults>60_surgical-15min: 11.6±7.6%, Adults<60_FFP2-30min: 12.1±4.5%, Adults<60_surgical-30min: 9.3±4.1%) indicate ascending deoxygenation and hypercarbia. Secondary changes (p-value≤0.005) to hemodynamic parameters (e.g. MAP: Adults>60_FFP2-15min: 9.8±10.4%) were found. Exhalation of blood-borne volatile metabolites e.g. aldehydes, hemiterpene, organosulfur, short-chain fatty acids, alcohols, ketone, aromatics, nitrile and monoterpene mirrored behaviour of cardiac output, MAP, SpO_2, respiratory rate and pET-CO_2. Exhaled humidity (e.g. Adults>60_FFP2-15min: 7.1±5.8%) and exhaled oxygen (e.g. Adults>60_FFP2-15min: 6.1±10.0%$^{-}$) changed significantly (p-value≤0.005) over time.

Conclusions: Breathomics allows unique physio-metabolic insights into immediate and transient effects of face-mask wearing. Physiological parameters and breath profiles of endogenous and/or exogenous volatile metabolites indicated putative cross-talk between transient hypoxemia, oxidative stress, hypercarbia, vasoconstriction, altered systemic microbial activity, energy homeostasis, compartmental storage and washout. FFP2 masks affected more pronouncedly than surgical masks. Older adults were more vulnerable to FFP2 mask induced hypercarbia, arterial oxygen decline, blood pressure fluctuations and concomitant physiological and metabolic effects.

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Ronja Weber Wednesday 15, Room 27, 14h50

University Children's Hospital Zurich, Division of Respiratory Medicine, Zurich, Switzerland (pediatric exhalomics group)

Asthma in one breath: Metabolic signatures for allergic asthma in children by online breath analysis

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Background: We hypothesized that the breath of children with allergic asthma contains an informative signature of metabolites that differentiates them from healthy children. Using secondary electrospray ionization high-resolution mass-spectrometry (SESI-HRMS), we aimed to identify a set of relevant VOCs, assess their biological connections and potential role in the pathophysiology of asthma.

Methods: Breath analysis was performed on an AB Sciex TripleTOF 5600+ HRMS (m/z range 50 - 500, mass accuracy < 2 ppm) coupled to a Super SESI ion source. A combination of data extraction and machine learning was used to isolate discriminative features and assess the predictive power of breath profiles. Putative compound identification was performed based on the analysis of MS2 spectra from breath, subgrouping into metabolic pathways and chemical families, screening for further relations based on the exact masses and a literature search.

Results: We acquired breath samples from 48 children with allergic asthma and 56 healthy controls aged 5 to 18 years. 375 m/z-features were significantly different between the two groups, 133 of which were putatively identified. Lysine degradation, tyrosine metabolism, 2-oxocarboxylic acid metabolism, fatty acid metabolites and monosaccharides were significantly upregulated in the asthmatic group, whereas arginine and proline metabolism, linoleic acid metabolism, aldehydes and fatty amides were elevated in the healthy cohort. Supervised machine learning resulted in an area under the curve (AUC) of 0.85 (95% CI: 0.74 - 0.94) for classifying asthmatics vs. healthy.

Conclusions: We report a large amount of tentatively identified exhaled compounds discriminating children with allergic asthma from healthy controls. Many of those can be mapped to rich metabolic pathways and chemical families that might be connected to pathophysiological mechanisms. A subset of the identified markers also has the potential for diagnostic applications.

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Sarah Dowling Tuesday 14, Auditorium, 15h10

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A clinical investigation into the ability of lung impaired subjects to provide screening and evidential breath specimens

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(3) CSTAR, Centre for Support and Training in Analysis and Research, School of Public Health, Physiotherapy and Population Science, Woodview House, University College Dublin, Belfield, Dublin 4, Ireland

Background: In the enforcement of drink driving laws failing to provide a breath specimen for alcohol analysis when requested by a Police Officer is an offence in many countries. Some drivers claim that a lung disease prevented their ability to be successful. These studies aimed to investigate the relationship between the presence of a lung disease and the ability to provide a successful breath specimen, both screening (roadside) and evidential.

Methods: Pulmonary function tests (PFT) were carried out on volunteers from outpatients of the pulmonary laboratory in St Vincent's University Hospital, Dublin and a control group with no underlying lung disease. After the PFTs all volunteers were asked to provide breath specimens using the screening device Dräger 6510 & then with an evidential breath analyser, the EvidenzerIRL.

Results: Using the Dräger only one participant (a female) failed to provide a sufficient specimen. For the EvidenzerIRL 14 (24%) out of 58 lung disease volunteers failed to provide a breath specimen, no one from the control group (n = 19) was unsuccessful. Thirteen females and one male volunteer could not successfully provide. A significant difference was found between the median age of successful (62.2 yrs) and unsuccessful (69.2 yrs) lung disease volunteers. Only one PFT, percentage predicted of Forced Expiratory Volume in 1 second (%FEV1), had a significant difference between the mean of successful (86.6%) and unsuccessful (66.5%) lung disease volunteers.

Conclusions: For the Dräger with only one unsuccessful participant, the presence of a lung disease did not indicate if a driver would be unsuccessful however all participants were free from infection and the participants with a lung disease were stable at the time of testing.

For the EvidenzerIRL female volunteers were found to be more likely to fail to provide than male volunteers. A subject with lung disease was more likely to be successful than unsuccessful. FEV1 was the only PFT found that could indicate if a lung impaired driver would fail to provide however there was some overlap seen between successful ad unsuccessful participants. Increasing age of lung disease participants also increased the chances of failing to provide breath specimens. Subjects' effort and operators' guidance through the process were found to be crucial parts to a successful outcome..

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Yoni E. van Dijk Tuesday 14, Room, 15h30

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Analysis of metabolites in exhaled breath for the phenotyping of eosinophilic asthma in children

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Background: For identifying eosinophilic asthma (EA), sputum eosinophil levels are currently considered the 'golden standard'. However, sputum collection is time-consuming and uncomfortable for children, therefore identifying additional non-invasive biomarkers could improve and simplify diagnostic workup. We hypothesized that the analysis of exhaled volatile organic compounds (VOCs) by gas chromatography-mass spectrometry (GC-MS) allows discrimination between EA and non-eosinophilic asthma (NEA) in pediatric asthmatics.
Methods: Exhaled breath samples were obtained according to ERS standards. Patients were recruited as part of SysPharmPediA cohort - a multi-center prospective pan-European observational study, in children with moderate to severe asthma. Participants were classified as EA/NEA using two absolute eosinophilic blood cell counts thresholds, 300 cells/µl (AEC300) and 400 cells/µl (AEC400). Through univariate Mann-Whitney U testing, we selected VOCs that differed significantly ($p<0.05$) between EA and NEA. Subsequently, we performed partial least square – discriminant analyses (PLS-DA) and calculated the area under the receiver operating characteristic curves (AUROCCs). Putative identification of identified VOCs was carried out using NIST libraries. With the retaining compounds, we repeated PLS-DA and calculated AUROCCs in a training (75%) and validation (25%) set.
Results: Complete data were available of 100 patients (11.7±3.6 year, 61% male, FEV1PB 101.5%), of which 69 (AEC300) and 52 (AEC400) were classified as EA. Univariate testing showed 31 (AEC300) and (AEC400) significantly different VOC fragments between EA/NEA. Nine of these fragments were found

at both thresholds and therefore selected for identification. Of the identified compounds, benzaldehyde and 1-methylenepropylbenzene retained after removal of potential contaminants. Final PLS-DA models resulted in AUROCCs of 0.67 (95%CI 0.53-0.81) for the training set and 0.68 (95%CI 0.39-0.97) for the validation set. No correlation was found for age, however the VOCs of interest did differ significantly between countries.
Conclusions: In this explorative study, we identified benzaldehyde and 1-methylenepropylbenzene as candidate biomarkers for discrimination between EA and NEA in pediatric asthmatics.

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Simonetta Capone Tuesday 14, Auditorium, 15h30

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Blood, urine and semen Volatilome analysis exploring health risk in contaminated areas in Italy

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Background: Two areas in central-southern Italy Land of Fires (LF) in Campania and Valley of Sacco river (VSR) are known to be contaminated sites, the first due to illegal fly-tipping and toxic fires, and the second due to an intensive industrial exploitation with dramatic consequences for environment and resident people [1]. Volatile Organic Compound (VOC) analysis is usually applied in pollution assessment by checking for toxic or harmful volatile compounds in air, but it has a great potential in Human Biomonitoring (HBM) studies [2].
Methods: The work contributes to HBM by a study conducted in LF and VSR areas on healthy young male population by two methods: a) Headspace Solid Phase MicroExtraction (HS-SPME) followed by Gas Chromatography-Mass Spectrometric detection (GC-MS); b) a semiconductor gas sensor array trained by SPME-GC/MS. Exogenous VOCs and their derivatives, metabolized by cells, were valued into specific body fluids (blood, urine and semen).
Results: Statistical analysis allowed to discriminate the two contaminated areas and identify those compounds which significantly contribute to the two areas classification. Some of these compounds are toxic and found prevalently in Valley of Sacco River samples, correspondingly to sperm analysis results for young men living in this zona worse than those living in Land of Fires. The sample fingerprinting by a gas sensors system allowed to discriminate the different contamination of the two areas and was able to predict the chemical concentration of several VOCs identified by GC/MS [3,4].
Conclusions: This work provides an untargeted but complete profile of the characteristic VOCs present in the considered biofluids by a standard GC/MS-based analytical analysis as well as a global fingerprint by a modern analytical tool as the e-nose based on gas sensors. The general perspective is a long-range focus on exploiting human biomonitoring approaches to discover unknown biomarkers and develop individual health risk predictors for humans.

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Alexander Möller Tuesday 14, Room 27, 15h30

University Children's Hospital Zurich, Division of Respiratory Medicine, Zurich, Switzerland (pediatric exhalomics group)

Asthma diagnosis in children by real-time breath analysis

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Background: Quality of allergic asthma diagnosis in children is critically poor and hence can lead to misdiagnosis, resulting in under/over treated children. Better, quick and accurate diagnostic tools that could help medical doctors are urgently needed. Breath-analysis has the ability to solve this problem. Here, we show that proton-transfer-reaction mass spectrometry (PTR-MS) could be used with children to detect metabolites in exhaled breath specific to allergic asthma and predict the disease based on the metabolic profiles.

Methods: Exhaled volatile organic compounds (VOC) of 53 children with allergic asthma and 50 healthy children aged 12.8 ± 3.1 years were collected with the Vocus-PTR-MS (TOFWERK AG, Switzerland). Mass spectra were acquired in the m/z range 50 - 300 with the standard H3O+ and the novel NH4+ modes (mass resolving power: 10000 m/Δm). A combination of dimensionality reduction and feature selection techniques were used to find biomarkers which could potentially be used to support asthma diagnosis. Supervised machine learning was used to assess the predictability of the biomarker profiles.

Results: We isolated 106 out of 436 m/z-features which could be used to define exhaled breath signatures specific to allergic asthma. The majority of the m/z-features could be chemically annotated and identified. When assessing the classification ability of the exhaled features with supervised machine learning in a 10 times repeated 10-fold cross-validation, we recorded an average AUC of 0.86 (95% CI: 0.75 - 0.95).

Conclusion: We conclude that the rapid and noninvasive exhaled breath collection using a Vocus-PTR-MS, could potentially be applied in a clinical setting for the diagnosis of allergic asthma in children. Since the breath profiles are recorded directly during exhalation a trained model could be deployed with further optimization returning the diagnosis result in almost real-time.

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Joris Meurs Tuesday 14, Auditorium, 15h50

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Non-invasive monitoring of participants during a multi-day walking event. Two case studies of the Nijmegen Four Days Marches

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Background: Non-invasive monitoring offers great potential for tracking metabolic changes caused by external stimuli. Two cohorts of participants were investigated during the multi-day walking event Nijmegen Four Days Marches of 2018 and 2019. The aim of these studies was to develop and validate in situ a breath sampling protocol and to identify variations in the breath volatile organic compound (VOC) profile as a result of exercise in combination with a cholesterol-lowering drug (statin) use and inflammatory bowel disease (IBD), respectively.

Methods: Proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) was used on-site to measure the exhaled breath profile of included participants. In both studies no restrictions were placed on the participants.

Each participant gave a duplicate breath sample. Breath samples were collected before the start of the event (Day 0), on the first three days post-walking (Day 1-Day 3), and on the second day pre-walking (Day 2 am). The sampling setup included a commercial breath sampler (Loccioni) in combination with a CO_2 trigger implemented to aid in the data analysis (Figure 1).

Advanced multivariate analysis techniques were used (multilevel partial least squares discriminant analysis (MPLS-DA) and ANOVA simultaneous component analysis (ASCA) to deal with the experimental design and investigate how different stimuli affect the breath profile, as well as individual VOCs.

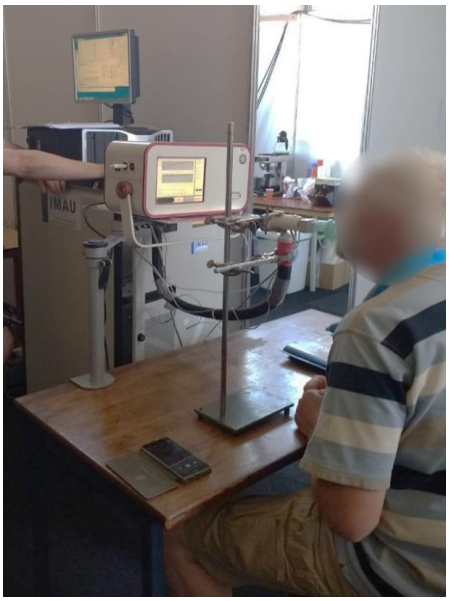

Fig. 1. On-site breath sampling during the 2018 edition of the Four Days Marches.

Results: We developed and validated a robust and reliable on-line protocol for breath sampling and analysis with PTR-ToF-MS for use in a field campaign, without controlling any external factors, such as food and drinks consumption. The breath collection/analysis was performed within 2 minutes with reproducible results (Lin's concordance correlation coefficent > 0.9). In both studies, a cluster of short-chain fatty acids (SCFAs) including acetic acid, butanoic acid and propionic acid were identified in exhaled breath as potential indicators of gut microbiota activity relating to exercise and drug use/IBD.

Statin use significantly reduced the breath SCFAs compared to the control group. In addition, butyric acid was identified as a potential marker to monitor exercise-induced inflammation.
Conclusions: Non-invasive montoring of exhaled breath VOCs during a field campaign with PTR-ToF-MS in combination with adeguate statistical analysis tools offer great insight into the effect of external stimuli on metabolic response.

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Andrei Malinovschi Tuesday 14, Room 27, 15h50

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Exhaled and nasal nitric oxide in clinical guidelines

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There has been a long way for fractional exhaled nitric oxide (F_ENO) to come into the clinical guidelines from the discovery of increased levels of FENO in asthma. A high cut-off >40 ppb (to compromise regarding sensitivity and specificity) or >50 ppb (for specificity >90%) is now recommended to support an asthma diagnosis in the recent European Respiratory Society (ERS) guidelines [1]. There is probably clinical information even in values below these cutoffs, but we need adequate reference values accounting for gender, age, smoking and IgE sensitization. For this purpose, a Global Lung Function Initiative (GLI) task force is currently performing such a work. Regarding treatment, the American Thoracic Society (ATS) clinical guidelines evaluated if F_ENO testing should indicated to optimize asthma treatment in patients considered to be put on treatment and concluded that F_ENO is beneficial and should be used in addition to usual care [2]. F_ENO is part of the definition of type 2 inflammation signature where a value of F_ENO >20 ppb is one of the hallmarks in patients with difficult to treat asthma or severe asthma on treatment. New biological treatments, such as dupilumab (anti-interleukin-4α), are indicated in patients with severe asthma with increased F_ENO and/or blood eosinophil counts [3]. Nasal nitric oxide (FnNO) has recently been reviewed by an ATS technical standard [4]. The current recommendations are to use FnNO as a screening method for of the diagnosis of primary ciliary dyskinesia (PCD) in subjects with clinical suspicion. Chemiluminescence devices are recommended to be used for aspiration from one nostril at a time while exhaling through the mouth against a resistance to close velum. Average concentration from each nostril is used to calculate NO output by multiplying this with the flow sampling rate. Values below 77 nL/min are highly sensitive and specific for PCD. FnNO is now available in devices with electrochemical sensors. However, the use of these devices for nasal NO need to be validated.

Conclusion: Since the discovery of high FENO in the exhaled gas in asthma patients it has taken more that 25 years to get this simple breath test into clinical guidelines. Generation of reference values for FENO and validation of electrochemical sensors for FnNO can hopefully further increase the clinical utility of the methods.

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Wednesday 15

Session 13 Young scientist session I

Chair: Dorota Ruszkiewicz, Tommaso Lomonaco

Session 14 Young scientist session II

Chair: Franziska Lochmann, Veronika Ruzsanyi

Session 15 Skin volatilome and other stories

Chair: Aoife Morrin, Tobias Bruderer

Hannah Schanzmann Wednesday 15, Auditorium, 10h10

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Ion mobility and mass spectrometry in combination with gas chromatography for the detection of nosocomial infections: first results

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Background: Hospital-acquired infections are one of the greatest challenges in inpatient care. They lead to increased mortality, a longer length of stay, higher treatment costs, and thus a socioeconomic burden. Nosocomial pneumonia is considered the most common cause of death among hospital-acquired infections [1]. One of the main problems is the lack of knowledge of the causal pathogen, which cannot be targeted without long-lasting microbiological diagnostics or MALDI TOF-MS. This can take between two to four days [2].
Methods: Therefore, a technology for targeted detection of nosocomial infections needs to be developed. The InosIn project's aim is to identify pathogens based on their microbiological volatile organic compound (mVOC) profiles in exhaled air. Ion mobility spectrometer coupled with gas chromatographic pre-separation (GCxIMS) allows direct breath sampling and measurements at the patient's bedside. Therefore, a mobile GCxIMS was set up, that needs to be validated for mVOC analysis. Since the identification of mVOCs is important to infer the specific metabolism of the pathogens, a benchtop system consisting of a thermodesorption (TD) gas chromatograph with a mass spectrometer (MS) is established. In addition, an IMS was introduced as a second detector in the same flow line using a flow splitter. The coupling of TD-GC-MSxIMS has been built up for the first time.
To find specific metabolites for selected known pathogens, the headspace of bacterial reference cultures grown on plates is measured using a self-developed sampling chamber.
Results: An exact correlation of the retention times of signals from reference substances between the two detectors (IMS and MS) was achieved. Thus, a database of more than 30 relevant substances for MS and IMS could be created. Initial measurements of selected bacterial cultures demonstrate, that especially the high detection power of the IMS enables the detection of pathogen-specific VOC patterns.
Conclusions: With the TD-GC-MSxIMS coupling, a powerful tool was created for the identification of mVOCs in the headspace of bacterial cultures. The next steps will be to expand the pathogen panel and apply multivariate statistical tools to analyze the complex data sets. The further aim is the comparison of patients' breath data with the results of the in vitro studies.

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Daria Slefarska-Wolak Wednesday 15, Room 27, 10h10

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Volatilomic footprints of AGS-1, SNU-1, CLS-145 and HGC-27 gastric cancer cell lines

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Background: Analysis of volatile organic compounds (VOCs) emitted by the human body opens up new perspectives for gastric cancer screening. Recent studies suggest that VOCs form chemical signatures that express distinct and immediate changes when various abnormal processes, including cancer, occur in the human body. The strategic aim of this study is to pinpoint the volatilomic signatures of four gastric cancer cell lines and to highlight possible differences between their volatilomic patterns.
Methods: Gas chromatography with mass spectrometry detection (GC-MS) and head-space needle trap extraction (HS-NTE) as the pre-concentration method were used to profile VOCs consumed and released by four human gastric cancer cell lines, AGS-1, CLS-146, HGC-27 and SNU-1, and two non-tumorigenic cell lines, HSEC and GES.
Results: Twelve VOCs were found to be consumed (e.g., benzaldehyde, 2-pentylfuran and DMDS) and fifteen released (e.g., ethyl α-methylbutyrate, 2-nonanone and 2-ethyl-1-hexanol) by the CLS-145, HGC-27 and HSEC cell lines. Furthermore, ten VOCs were found to be metabolized (e.g., 2-ethylfuran, 6-methyl-2-heptanone and ethyl benzoate) and thirty-five volatiles emitted (e.g., 3-pentanone, 1,1-diethoxyethane and cyclohexanol) by AGS-1, SNU-1 and GES cell lines.
HGC-27 and AGS-1 cell lines are characterized by upregulated production of ketones with an odd number of carbons. CLS-145 and SNU-1 cell lines exhibit increased production of esters and downregulated production of alcohols.
Conclusions: Each of the gastric cancer cell lines has its individual metabolic pattern. The results from this study provide evidence that gastric cancer alters the VOC profiles of gastric cell lines. Thus, our study demonstrates that VOC analysis of different human excretions, such as breath or urine, has potential for use as a non-invasive tool for the diagnosis of gastric cancer.

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Lorenzo Petralia Tuesday 14, Auditorium, 10h30

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A novel methodology towards the functional location of inflammation in eosinophilic asthma

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Background: In the T helper 2 (Th2)-high asthma subtype, the production of nitric oxide (NO) is greatly enhanced from the sites of inflammation, and the lung is also more inhomogeneous in terms of gas exchange. Over the past decades, researchers have improved measurements and models for the fraction of exhaled NO (FeNO), thereby leading to a better understanding of the complexities of this asthma endotype [1,2]. Although Th2-high asthma is generally well-controlled with inhaled corticosteroid (ICS) treatment [3], a substantial subgroup of these patients are ICS non-responders [4]. This results not only into significant healthcare costs but also in unnecessary high ICS dosage with the risk of side effects. It is crucial to non-invasively assess the location of inflammation and predict adherence to ICS therapies.
Methods: To this end, we developed a novel methodology based on combining time resolved FeNO profiles with highly precise measurements of respiratory gas exchange that assess functional inhomogeneity in the lung.
For each patient we acquire multiple simultaneous FeNO and CO_2 expirograms, followed by a N_2 washout scan utilising our laser-based sensors [5,6]. Specifically, from the gas-exchange data of each individual, our mechanistic pulmonary model evaluates the particular type and degree of inhomogeneity; FeNO production is therefore examined in the context of an inhomogeneous lung. The patient-specific lung parameters are then used to produce bespoke simulation of the CO_2 and FeNO profiles with which to interpret the measured data.
Results: Within a collaboration between the departments of Chemistry, Physiology and Genetics, and the Nuffield Department of Medicine in Oxford we are performing a pilot study in clinic to show the applicability of this novel methodology. We are in the process of collecting this type of data on asthmatic patients at the Respiratory Medicine Unit of the John Radcliffe Hospital in Oxford.
Conclusions: Our aim is to ascertain whether the responsiveness to ICS therapy in Th2-high asthma is related to site of FeNO production in the airways. This non-invasive and unique methodology could improve the monitoring of airway inflammation for the diagnosis and treatment of eosinophilic asthma.

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Eline Janssens Wednesday 15, Room 27, 10h30

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Breath biomarkers for pleural mesothelioma: An external validation study

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Background: To diagnose malignant pleural mesothelioma (MPM) in at-risk asbestos-exposed (AEx) individuals at an early stage, a breath test by ion mobility spectrometry was previously developed by our research group [1]. However, before clinical implementation of this model, it is essential to test its robustness and reproducibility through external validation. So the aim of this study was to assess the predictive performance of the model using an independent group of participants and to update the model if necessary.

Methods: Breath samples of 47 MPM patients and 67 at-risk AEx controls were analyzed as previously described [1]. Based upon a specific combination of volatile organic compounds (VOCs) in the exhaled breath samples, the class of the participants (MPM/control) was predicted by the model. The degree of agreement between the predicted and true class of the participants was determined in terms of sensitivity, specificity and accuracy.

To update the model, a new lasso regression was fit to the validation samples using the same preselected subset of VOCs as predictor variables. Predictive performance of the updated model was estimated through internal validation by leave-one-out cross-validation.

Results: see Fig.1.

Conclusions: External validation showed poor performance of the

(A)

	External validation original model	*Internal validation updated model*
Sensitivity	0.53 (0.39 - 0.67)	0.85 (0.73 - 0.93)
Specificity	0.33 (0.23 - 0.44)	0.62 (0.51 - 0.72)
Accuracy	0.41 (0.32 - 0.50)	0.71 (0.62 - 0.78)

(B)

	MPM			AEx		
	Discovery set	*Validation set*	*p-value*	*Discovery set*	*Validation set*	*p-value*
N	52	47		100	76	
Gender (M/F)	43/9	39/8	1[a]	98/2	71/5	0.241[a]
Age	66.43 ± 8.31	69.99 ± 6.36	0.018[b]	55.72 ± 6.62	55.73 ± 9.47	0.991[b]
BMI	25.29 ± 3.10	25.40 ± 3.56	0.876[b]	27.59 ± 3.84	27.25 ± 4.19	0.585[b]
Smoke status (never/current/ex)	19/5/28	22/3/22	0.561[a]	34/22/44	33/10/33	0.235[a]
Packyears	2.65 (0.00-14.55)	5.25 (0.00-20.00)	0.962[c]	5.80 (0.00-24.15)	1.50 (0.00-15.00)	0.170[c]

Figure 1: **(A) Model performance.** Values presented with 95% confidence interval. **(B) Participant characteristics of the original discovery set and external validation set.** Values presented as n, mean±SD or median (Q1-Q3).[a]Fisher's exact test; [b]T-test; [c]Mann-Whitney U test.
AEx = asbestos-exposed individuals; MPM = malignant pleural mesothelioma patients.

original model, which could be due to several factors such as sampling location, time, interobserver variability or instrumental wear. Updating the model improved its performance, emphasizing the added value of the originally selected VOCs, but would ideally need revalidation. This study highlights the necessity of external validation within biomarker research to ensure reproducibility.

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Ning Sun Wednesday 15, Auditorium, 11h20

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Profiling volatile organic compounds from human plasma using GC×GC-ToFMS

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Background: Volatile organic compounds (VOCs), originating from various metabolic activities, can be detected and identified after elimination in breath, urine, feces, skin, etc. They are candidate biomarkers specially for the diagnosis and monitoring of lung disease because they originate in the respiratory tract where the breath is in close contact[1]. Up to 2021, nearly 1500 VOCs have been reported in the breath, while only 379 have been found in blood, even though VOCs pass through the lungs from blood circulation[2]. Current studies focusing on blood VOCs are limited to monitoring targeted pollutants[3], blood storage or decomposition[4], and biomarker studies identifying VOCs that can distinguish between healthy and disease groups[5]. However, a comprehensive characterization of human blood VOCs collected for routine diagnostic testing is lacking.
Methods: 97 blood samples were obtained and separated into training (n=72) and validation (n=25) set. VOCs were extracted from blood-derived plasma using solid-phase microextraction and analyzed using two-dimensional gas chromatograph tandem time of flight mass spectrometry (GC×GC-ToFMS). Chromatographic data were aligned, and putative compound identities were assigned via spectral library comparison. Statistical analysis was performed in R.
Results:

- Pan volitalome of human blood comprised with 401 molecules, of which, 34 were present in all training samples.
- The core molecules were dominated by aliphatic hydrocarbons, aromatic compounds, and carbonyl compounds with a validation accuracy of 99.9%.
- Putative names were assigned to seven of the core molecules with a normalized area variance less than 0.05.

Conclusions:

- Our core molecules were consistently present in all samples irrespective of age, sex or disease status.
- Our data describe the baseline VOC profile in human blood and can complement future studies of disease biomarkers in blood or breath.

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Kathleen Zwijsen Wednesday 15, Room 27, 11h20

Laboratory of Experimental Medicine and Pediatrics, University of Antwerp

Analysis of VOCs in exhaled breath as screening method for malignant pleural mesothelioma in an asbestos-exposed population

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Background: Malignant pleural mesothelioma (MPM) is an aggressive cancer of the serosal lining of the lungs and chest cavity, predominantly caused by historical asbestos exposure. Due to aspecific and late symptoms, MPM is characterized by an advanced-stage diagnosis, resulting in a 5-year survival rate <5%. However, early diagnosis is believed to improve patient outcome. Currently, no diagnostic or screening tools are available. Therefore, our research group investigated exhaled breath as this can easily be obtained non-invasively, without discomfort and contains volatile organic compounds (VOCs), which are considered biomarkers for multiple (patho)physiological processes [1]. A breath test was developed and differentiated asbestos-exposed persons from MPM patients with 87% accuracy. However, before being implemented in the clinic, clinical utility of the test must be determined [2].
Methods: The aim of the current research is to assess the clinical utility of this breath test for early detection of MPM through VOC analysis. Therefore, asbestos-exposed individuals will undergo the test annually using multicapillary column/ion mobility spectrometer (MCC/IMS) and the individual risk of MPM will be estimated. A correlation between the breath profiles and paired low-dose CT-scan will be done to match test outcome with radiological findings.
Results: The study initiated in October 2021 and tests will be repeated annually. The first screening round is currently ongoing. First results of the CT scans and their correlation with VOCs are expected Q3 2022.
Conclusions: Analysis of VOC patterns in exhaled breath of asbestos-exposed individuals can possibly be used as a screening tool for early detection of MPM patients. As approximately 150 participants are willing to undergo breath sampling during a 4-year period, screening a larger population with the breath test is feasible. This could lead to the breath test being rolled out as a screening tool for MPM.

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Iris van der Sar Wednesday 15, Auditorium, 11h40

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Unsupervised clustering of electronic nose data in patients with sarcoidosis

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Background: Sarcoidosis is a multisystem granulomatous inflammatory disease that can affect almost any organ. Due to its heterogeneous clinical presentation and disease course, it can be challenging to diagnose and treat. We previously found that electronic nose (eNose) technology distinguishes sarcoidosis from healthy controls very accurate, without distinctive differences within sarcoidosis groups [1]. We aimed to evaluate whether unsupervised clustering of eNose data results in clinically relevant phenotypes.

Methods: In a cross-sectional single center study, exhaled breath of sarcoidosis patients was analyzed using an eNose (SpiroNose). Unsupervised partitioning around medoids cluster analysis was applied to eNose sensor data. Consensus clustering method was used to assess cluster stability [2]. Comparison of clinical parameters between clusters was performed using one-way ANOVA, Kruskal-Wallis, and $\chi 2$ tests.

Results: 252 patients were included. Two clusters showed the highest stability. Clinical characteristics of patients in cluster 1 (n=69) did not differ significantly from patients in cluster 2 (n=183) (Table 1).

Conclusions: Two distinctive sarcoidosis patient clusters were found based on eNose sensor data. However, selected clinical characteristics did not differ between the clusters. This is in line with previously published supervised analyses of this cohort, that showed detection of sarcoidosis independent of clinical phenotype [1].

Table 1. Clinical characteristics of clusters identified

	Cluster 1 (n=69)	**Cluster 2** (n=183)	*p-value*
Male	35 (50.7)	99 (54.1)	*0.74*
Age	54.38 (11.4)	52.64 (11.4)	*0.28*
BMI, kg/m^2	27.67 (5.0)	27.91 (4.6)	*0.73*
Smoking status			*0.76*
Ex-smoker	22 (31.9)	61 (33.3)	
Never smoker	44 (63.8)	110 (60.1)	
Smoker	3 (4.3)	12 (6.6)	
Immunosuppressant use	29 (42.0)	92 (50.3)	*0.31*
FVC, % of predicted ~	88.96 (18.2)	87.47 (15.9)	*0.53*

DLCO, % of predicted #	79.62 (16.2)	75.68 (19.1)	*0.14*
Serum sIL-2R level, U/ml $	450.5 [304.2-640.0]	459.0 [339.5-622.8]	*0.61*
Serum sIL-2R elevated $	23 (67.6)	66 (67.3)	*1.00*
Pulmonary involvement	59 (85.5)	164 (89.6)	*0.49*
Pulmonary fibrosis *	10 (21.3)	42 (27.5)	*0.51*
Number of organs involved			*0.05*
1 organ	9 (13.0)	15 (8.2)	
2 organs	24 (34.8)	72 (39.3)	
3 organs	24 (34.8)	59 (32.2)	
4 organs	5 (7.2)	31 (16.9)	
>4 organs	7 (10.1)	6 (3.3)	
Time since diagnosis, months ^	60.0 [31.0-113.5]	74.0 [28.0-146.5]	*0.29*
Comorbidity			
Diabetes mellitus	6 (8.7)	18 (9.8)	*0.97*
OSAS	10 (14.5)	17 (9.3)	*0.34*
Asthma	5 (7.2)	12 (6.6)	*1.00*
Cardiovascular disease	18 (26.1)	44 (24.0)	*0.86*

Categorical variables are displayed as number and percentage of total cluster patients; continuous variables as mean value with standard deviation or median with interquartile range. $p<0.05$ was considered as statistically significant. Missing values are handled as missing. ~ Missing in n=9 patients. $ Missing in n=120 patients. A sIL-2R value >550 U/ml is considered elevated. # Missing in n=20 patients. * Missing in n=52 patients (no HRCT scan performed). ^ Missing in n=2 patients. BMI = body mass index; DLCO = diffusing capacity for carbon monoxide; FVC = forced vital capacity; OSAS = obstructive sleep apnoea syndrome; sIL-2R = soluble IL-2 receptor.

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Federico Vivaldi Wednesday 15, Room 27, 11h40

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A low cost setup for dispensing internal standard into needle trap microextraction devices for a reliable breath and environmental analyses

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Background: The development of successful breath tests relies on the development of robust and easy use analytical procedures. Sampling by needle trap microextraction (NTME) is becoming more and more popular as it guarantees analytical performances similar to common SPE methods using just 10 mL of sample. When working with humid gaseous samples, the amount of water vapor collected in a needle trap device (NTD) may vary from sample to sample and decrease during storage, thus impacting desorption efficiency and analyte recovery. Recently, we demonstrated that the use of internal standard (IS) reduces the overall NTME method variability [1]. As next step, we propose here an analytical procedure for the reliable production of gaseous standard mixture of IS and its reproducible addition into NTDs.

Methods: A 1 L silco-steel canister was cleaned with methanol and dried under vacuum (30 mmHg) at 60 °C overnight before being spiked with an aliquot (10 µL) of a methanolic solution of 8D-Toluene (2.9 mg/mL). The canister was filled with pure nitrogen at 37 psi and kept at 40 °C for 30 min before connecting it to a mass flow controller (MFC) for the accurate delivery of IS to NTDs. The system was operated through an ATMega328-pu for the precise control and reading of the MFC. The readout of the system was achieved through Bluetooth and an OLED display.

Results: Flow rate and volume could be set either through a rotary encoder mounted onto the controlling board or through a dedicated android app. The variability of the flux in the range 5 – 200 mL/min was lower than 1 %. At the same time, a SIFT-MS system was employed to evaluate the variability (< 3 %) of the IS during the delivery through the MFC. No carry-over was detected in the system after the delivery of the IS. A GC-QqQ was used to analyze the NTDs obtaining intra and inter-day precisions of 10 and 15 %, respectively (n = 8 and n = 24). The concentration of 8D-Toluene in the canister was stable up to a month at room temperature.

The procedure was employed in a clinical setting to collect ambient air and breath samples from 20 patients suffering from chronic heart failure and undergoing an incremental exercise test using a cycloergometer.

Conclusions: Our low cost system allowed the addition of the internal standard in the NTDs with high accuracy and reproducibility.

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Nynke Wijbenga Wednesday 15, Auditorium, 12h00

Department of Respiratory Medicine, Erasmus Medical Center, Erasmus University, Rotterdam, the Netherlands

Unsupervised clustering of electronic nose data in patients with sarcoidosis

Nynke Wijbenga (1)(5), Rogier A.S. Hoek (1)(5), Bas J. Mathot (1)(5), Leonard Seghers (1)(5), Catharina C. Moor (1), Joachim G.J.V. Aerts (1), Daniel Bos (2)(3), Olivier C. Manintveld (4)(5), Merel E. Hellemons (1)(5)

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Background: Lung transplantation is a life-saving treatment option for some patients with end-stage lung disease. Chronic lung allograft dysfunction (CLAD) negatively affects long-term survival of lung transplant recipients (LTR), but establishing the diagnosis can be challenging. Early diagnosis of CLAD could allow early intervention to halt progression. Electronic nose (eNose) technology could play an important role in early diagnosis of CLAD. We aimed to assess the feasibility and reliability of exhaled breath analysis using an eNose to detect CLAD in LTR.
Methods: In this cross-sectional study, exhaled breath of consecutive LTR with and without CLAD was analysed using an eNose (SpiroNose). CLAD diagnosis was made according the ISHLT criteria. The dataset was randomly divided into a training and (internal) validation set. Statistical analyses were

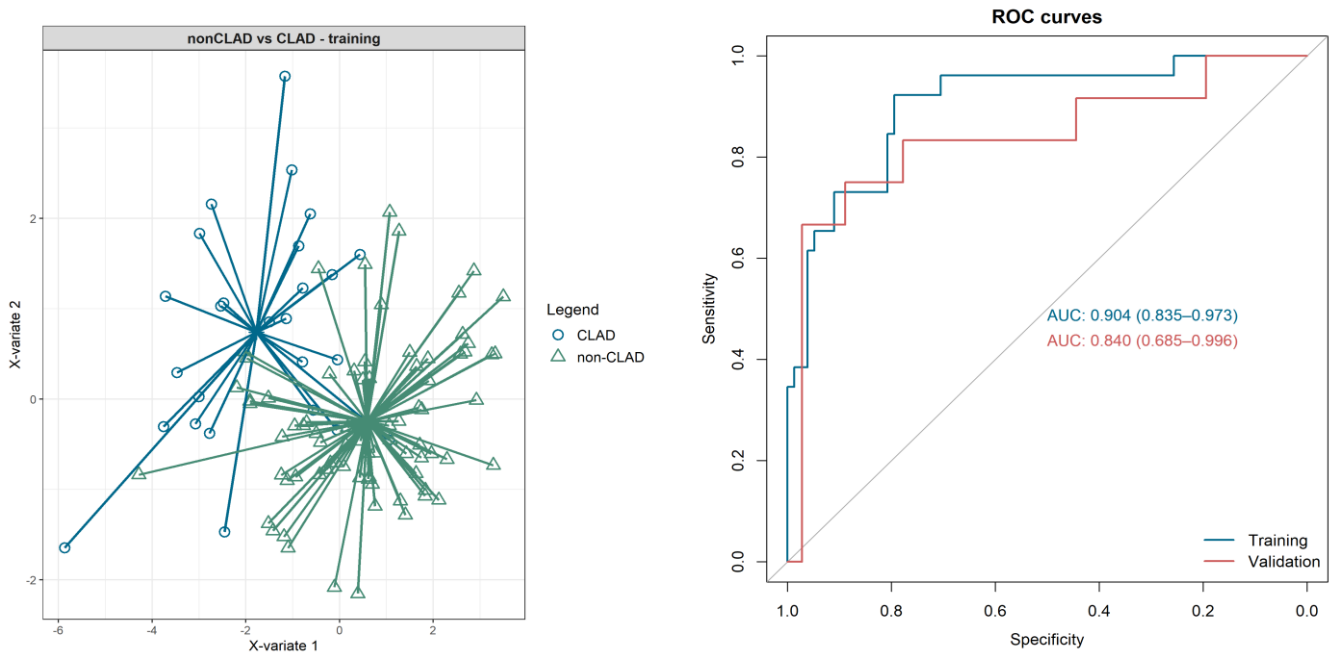

Fig. 1. Discrimination between LTR with and without CLAD. On the left the results of the PLS-DA model for the training set. On the right the corresponding ROC curves for both the training and the validation set.

conducted using partial least square discriminant analysis and receiver operating characteristics (AUC) analysis to assess differences in breathprint between LTR with and without CLAD.
Results: A total of 152 LTR (median age 60 years, 49% females, 86% transplanted bilaterally), of whom 38 with CLAD were included during outpatient follow-up. The training set consisted of 78 LTR without CLAD and 26 LTR with CLAD. The validation set consisted of 36 LTR without and 12 LTR with CLAD. In the training set the SpiroNose reliably discriminated between LTR with and without CLAD with an AUC of 0.90 (95% CI 0.84 – 0.97), a sensitivity of 92%, a specificity of 79%, and an accuracy of 83%. In the validation set the AUC reached 0.84 (0.69 – 1.00) with a sensitivity of 75%, a specificity of 89%, and an accuracy of 85%.
Conclusions: LTR with and without CLAD differ in breathprint. Exhaled breath analysis using an eNose is a very promising novel biomarker for CLAD enabling timely diagnosis. eNose technology could be an valuable addition to the diagnostic armamentarium for suspected graft failure in LTR.

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Aoife Morrin Wednesday 15, Room 27, 12h00

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Skin Volatilomics: Translating for wearable biodiagnostics for health monitoring

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For the last number of years, researchers have been looking at ways to undertake monitoring of health in non-invasive ways via the skin. While some exciting innovations in this field related to continuous or semi-continuous biomarker monitoring in sweat and interstitial fluid has been reported, our approach has focussed on capturing and analysing the volatile compounds coming from skin. The skin surface emits many classes of volatile organic compounds (VOCs), as well as ammonia, which are derived directly from glandular secretions and also from their interactions with resident skin bacteria. These VOC emissions have the potential to offer insights into cutaneous and systemic physiology. However, in general, and in contrast to breath volatile research, research into skin-emitted VOCs as they relate to pathological state is at an early stage. Skin volatile profiles of healthy individuals have been shown to be influenced by gender and other genetic traits. However, little consistency has been seen across studies with regards to the compounds observed, likely due to the wide variety of sampling and analysis approaches being used but also the highly variable nature of the skin matrix itself. More research is required to more deeply understand the healthy skin volatile profile and the impact of disease on this emission profile. Our work to date has focussed on a workflow we use to sample healthy participants skin volatiles and this talk will present our approach, discuss the findings, and finally its potential translation for wearable biodiagnostics.

Methods: This talk will introduce the workflow we implement for sampling and analysis of healthy participant skin volatiles using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). Measurements of skin surface pH and tissue dielectric constant were also collected with the participants VOC sample. The correlation of aspects of the processed skin volatile emission datasets with these measurements as well as participant age and gender were examined. Correlations identified in this work prompted the design of colorimetric wearable sensor platforms for monitoring skin health.

Results: Correlations between specific skin surface emission compounds and abundances (e.g. specific compound and compound class normalised abundances, total VOC abundance, etc) as detected by GC-MS were identified for factors including skin surface pH, gender and age. Work is ongoing to continue to collect participant data in this regard, the results of which will be presented in detail in the talk.

Conclusions: It would appear that certain VOCs being emitted from the surface of the skin correlate with other skin characteristics, e.g. gland distributions, skin surface pH, etc. It is interesting to harness this emission for the design of future wearable biochemical sensors for non-invasive health monitoring.

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Carmen Bax Wednesday 15, Auditorium, 12h20

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An experimental apparatus for e-nose breath analysis in respiratory failure patients

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Background: Nowadays, a key in the personalization of respiratory failure patients' management is providing bedside diagnostic tools [1]. Electronic noses (EN) represent an emerging tool for this purpose. By analysing endogenous Volatile Organic Compounds (VOCs) in breath samples, EN can phenotype respiratory disorders and improve diagnosis [2]–[5]. As EN application in respiratory failure patients is challenging, this work proposes a novel apparatus for exhaled breath sampling [6], [7].
Methods: It uses hospital medical air and oxygen pipeline systems to control the fraction of inspired oxygen, prevent contamination of exhaled gas from ambient VOCs and minimise the respiratory load imposed on patients. A commercial EN with custom MOS sensors was used to assess breath odour fingerprints. To collect data on tolerability and for a preliminary assessment of sensitivity and specificity, a feasibility study on 33 SARS-CoV-2 patients (25 with respiratory failure and 8 asymptomatic) and 22 controls was carried out. Boruta algorithm [8], was used to discriminate the most significant features to identify respiratory failure patients and controls breath odour fingerprints. Then, a classification model based on Support Vector Machine (SVM)[9] was implemented on selected features.
Results: All the patients well tolerated the proposed sampling system. The SVM model differentiated between respiratory failure patients and controls with an accuracy of 0.81 (area under the ROC curve), and a sensitivity and specificity of 0.92 and 0.68, respectively. The selected features were significantly different in SARS-CoV-2 patients with respiratory failure versus controls and asymptomatic SARS-CoV-2 patients ($p<0.001$ and 0.046, respectively).
Conclusions: The developed breath sampling apparatus proved suitable for respiratory failure patients, and results achieved within the feasibility study highlighted the potentialities of EN VOCs analysis for assessing lung disease severity and aetiology.

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Tobias Bruderer Wednesday 15, Room 27, 12h20

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A novel method to analyze sweat volatiles during fear stimulation with dynamic headspace extraction and comprehensive GCxGC high resolution MS

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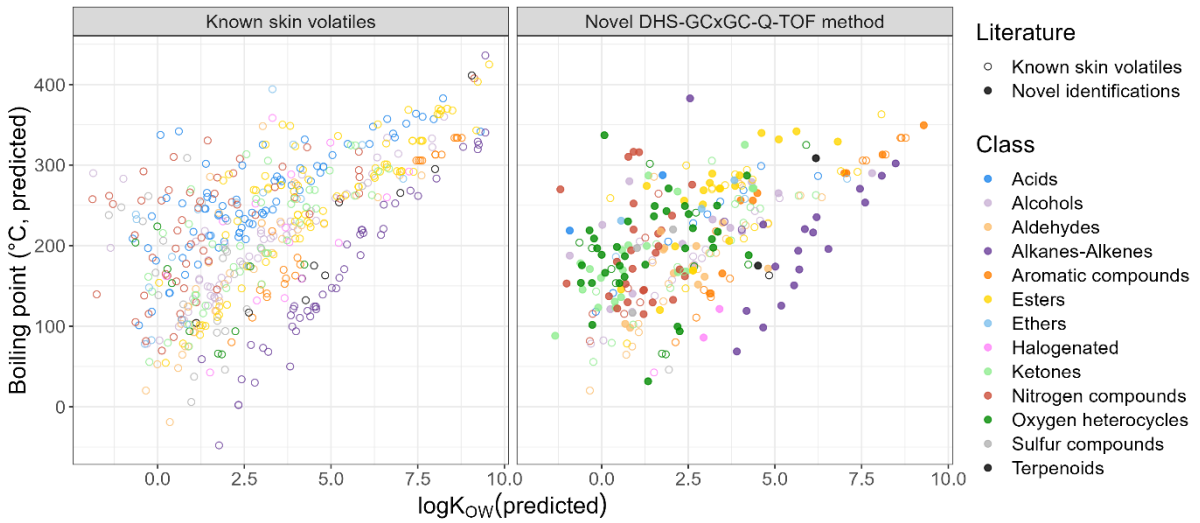
Background: Previous work has shown that body odour can affect the emotional state of the receiver [1]. Body odour could therefore be relevant for human experiments, particularly with focus on volatile organic compounds. One aim of the Potion project is to identify the relevant volatiles from sweat collected from participants during fearful or joyful emotional state [2]. We investigated differences in sweat volatile composition in a highly controlled study by fear stimulation with 40 participants
Methods: We developed a novel, comprehensive method for the analysis of sweat volatiles. Chemicals were collected with pre-treated pads, and were extracted, enriched, and trapped on Tenax GR tubes with dynamic headspace analysis (DHS, at 60°C), and analyzed by comprehensive two-dimensional gas chromatography (GCxGC) and time-of-flight mass spectrometry (TOF). Emotions were induced with virtual reality (VR) scenarios, and individuals were equipped with mobile sensors (EDA, ECG, respiratory rate).
Results: Emotional rankings showed a successful induction of fear, and significant differences in sensor features (EDA, ECG) in accordance with literature. We tentatively identified 311 compounds, assigned 38 class unknowns, and can report 15 true unknowns. This set of 364 compounds was monitored with high selectivity across samples. We could further narrow down the detected volatiles to a set of 287 relevant sweat volatiles compared to field blank pads (FC < 3.0, p.FDR < 0.05). Our method shows a good compound coverage with 581 known skin volatiles [3], with a perfect match for 116 compounds (Figure 1). Possible limitations are the detection of certain organic acids and a few very volatile compounds. Out of these compounds, we can report a subset of 24 sweat volatiles which were significantly increased during fear vs. relaxed condition (FC: 2.21±0.78, p.FDR.BH < 0.05, with paired t-



tests). Only five of these compounds have previously been reported as skin volatiles and only half of them have so far been detected in humans.
Conclusions: We can report a set of 24 volatile organic compounds which were significantly increased during fear which were detected with a novel, comprehensive DHS-GCxGC-TOF method for the analysis of human sweat samples.

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Franziska Lochmann Wednesday 15, Auditorium, 12h40

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Non-invasive CYP2C9 breath tests for predicting individual drug responses

Franziska Lochmann (1)(2), Aleksandar Nikolajevic (3), Jakob Troppmair (3), Chris A. Mayhew (1)(2), Veronika Ruzsanyi (1)(2)

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Background: The use of breath volatile biomarkers as predictors to individual drug response would significantly advance the field of personalized medicine, the benefits of which would be enormous. Extensive polymorphisms in a major group of drug-metabolizing enzymes, the cytochrome P450 family (CYPs), cause differences in metabolism. The enzyme CYP2C9 is responsible for metabolizing many crucial drugs, such as diclofenac ($C_{14}H_{11}Cl_2NO_2$), and food compounds, such as limonene ($C_{10}H_{16}$). Biomarkers produced by different substrates that are metabolised by CYP2C9 can potentially be used to quantify enzyme activity. By way of example, we present here details of an in vitro study that investigated the bioconversion of diclofenac in recombinant HEK293 cells overexpressing CYP2C9 and the competitive influence of limonene on its metabolism.

Methods: HEK293 (CYP2C9) and parental HEK293T cells were kept in DMEM containing 10% FBS, 1% PenStrep and 1% L-Glutamine in a humidified atmosphere containing 5% CO_2. Diclofenac sodium salt was dissolved in Milli-Q water, d(+)-limonene and 4'-hydroxydiclofenac in methanol. Calibration standards of diclofenac and 4'-hydroxydiclofenac (0.01 µM, 0.1 µM, 0.5 µM, 1 µM, 5 µM, and 20 µM) were prepared by serial dilution of the stock solutions with 50% acetonitrile (ACN). To prove that the products measured result from the bioconversion of the substrate parental HEK293T cells were used as control samples.

Results: Limonene is found to have an inhibitory effect on the bioconversion of diclofenac in the in vitro experiments. Increasing limonene levels continuously reduced the production of diclofenac's metabolite, 4'-hydroxydiclofenac (figure 1).

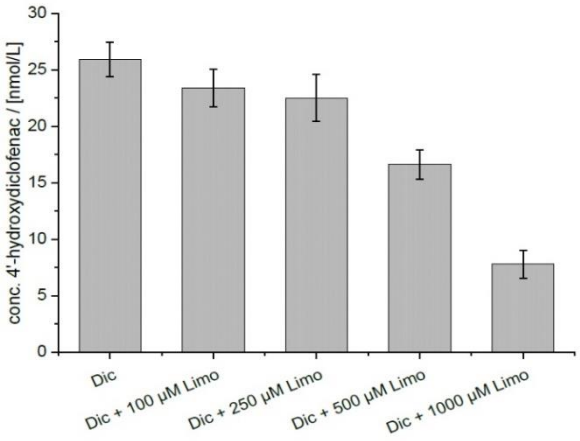

Fig. 1. Concentrations of 4′-hydroxydiclofenac after 4h bioconversion of HEK293 cells overexpressing CYP2C9 with a constant concentration of diclofenac (Dic) of 100 µM with increasing limonene (Limo) concentration.

Conclusions: Based on the successful conversion of diclofenac using the recombinant HEK cells, a workflow using a cell-based system for CYP2C9 activity has been established. Our plan is to implement this workflow for future studies to investigate substrate bioconversion by CYP2C9 and for other CYP isoforms, specifically with the aim to develop a non-invasive breath test for personalized medicine.

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Amy Worrall

School of Pharmacy and Bioengineering, Keele University

Wednesday 15, Room 27, 12h40

Predicting pathology: Examining the health of inner ear cells via VOC sampling to facilitate early intervention in age related hearing loss

Amy Worrall (1), Abigail Rutter (1), Nicholas Forsyth (1), David Furness (2), Falko Drijfhout (3)

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Background: Age related hearing loss (ARHL) affects most over 65, with current strategies (hearing aids/cochlear implants) unable to offer improvement with disease progression. Where inflammation of cochlear fibrocytes [1,2] is the leading pathology[3,4], regenerative interventions are possible[5,6,7]. These offer a favorable, long-term alternative to current treatments. However, to facilitate them, an early detection strategy for inflammation is required to enable timely intervention and monitoring post-treatment. This research presents a strategy wherein SIFT-MS may be used to monitor fibrocyte health non-invasively via relevant biofluids/culture headspaces.

Methods: Expanded murine fibrocyte cultures were characterized via ICC. Inflammation was induced via IL-1β, and confirmed by IL-6/IL-8 expression. Culture headspace was analysed using SIFT-MS (Transpectra Profile 3, H3O+ and NO+, m/z 1-180), and gas chromatography mass spectrometry (GC-MS) with solid phase micro-extraction (SPME). Statistical testing of SIFT-MS was performed via Mann Whitney U test and Kruskal-Wallis H test. Multivariate analysis (PCA) was used to identify sources of variation in SIFT-MS and GC-MS spectra.

Results: Fibrocytes were successfully expanded in culture with ICC confirming expected expression. SIFT-MS shows differences in acetaldehyde ($U(N=5)=2$, $z=-2.193$, $p=0.032$), butyric acid ($U(N=5)=2$, $z=-2.193$, $p=0.032$), benzaldehyde ($U(N=5)=0$, $z=-2.611$, $p=0.008$) and pyruvic acid ($U(N=5)=0$, $z=-2.611$, $p=0.008$) between media controls and cells. These compounds are seen in various mammalian biofluids and predominantly vary as a result of ethanol and glucose metabolism. SIFT-MS of inflamed cells shows differences between undosed and dosed cultures in key compounds, with singificance in acetaldehyde variation across conditions ($H(3)=8.641$, $p=0.034$). GC-MS corroborates SIFT-MS, showing similar compound variations. PCA shows variations attributable to key compounds. These results demonstrate inflammation detection via fibrocyte culture headspace.

Conclusions: These results represent a step forward in understanding of inner ear inflammaging and ARHL progression; showing the use of SIFT-MS for cochlear health monitoring. This work also represents an addition to knowledge of inflammatory associated VOCs, relevant to breath studies and beyond.

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Posters

Monday 13

Flash poster presentations 17h00 – 17h30

Poster session 17h30

Sunset on Lungarni Pisani, Pisa

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FLASH POSTER PRESENTATION SESSION 1

AUDITORIUM - FROM 17h00

Chair Dr. Pietro Salvo

Marek Jackowski	–	Protocols for colorectal cancer biomarkers
Mahya Khaki & Matthias G. Friedrich	–	The predictive value of the heart rate response to breathing maneuvers for significant coronary artery disease
Agapios Agapiou	–	VOCs and PM in confined environments
Tanja Zivkovic Semren	–	Workflow Development for Real-Time Exhaled Breath Analysis by Secondary Electrospray Ionization coupled to High Resolution Mass Spectrometry
Tara Lovestead	–	A pilot study to determine if THC can be detected in breath aerosols collected from legal market cannabis users with an impaction filter device
Marilena Giglio	–	Quartz-enhanced photoacoustic detection of ammonia in exhaled breath
Giuseppe Ferrandino	–	Pre-clinical exogenous volatile organic compounds (EVOCs) Probes screening and optimization for chronic liver diseases detection
Marieann Högman	–	Alveolar nitric oxide in COPD – a 2-year follow-up
Leo Rührmund	–	Data visualization for real time mass spec-based breath analysis in clinical setups
Cedric Wüthrich	–	Online SESI-HRMS breath analysis after a nutritional intervention challenge
David M. Fothergill	–	Exhaled breath condensate profiles of US Navy divers following prolonged hyperbaric oxygen (HBO) and nitrogen-oxygen (Nitrox) chamber exposures

FLASH POSTER PRESENTATION SESSION 2

ROOM 27 - FROM 17h00

Chair Dr. Denise Biagini

Ning Sun	–	A core breath profile of healthy non-human primates
Simonetta Capone	–	Analysis of urinary volatile organic compounds by electronic nose and GC/MS for prostate cancer diagnosis
Austin Meister	–	Detection of SARS-CoV-2 Omicron infection in exhaled breath from out-patients with mild respiratory symptoms
Francesco Segrado	–	Mass spectrometry profiling of exhaled breath of smokers to identify a signature related to tobacco use
David J. Mager	–	Towards targeted exhaled breath analysis for young children in CF care to detect bacteria in the lungs
Dominic Sandhu	–	Utilising computational methods to determine an idealised lung clearance index
Joris Meurs	–	Development and validation of a proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) method for analysis of short-chain fatty acids (SCFAs) in exhaled breath
Raj Attariwala	–	Correlation of breath and blood cannabis levels using custom-made breath sample collection and analysis method
Evangelia Sakkoula	–	Monitoring dietary status and cognitive functioning in children through exhaled breath analysis
Pritam Sukul	–	Recommended methods for safe breath analysis under highly infectious respiratory conditions
Sean W. Harshman	–	Investigation of an individual with low exhaled isoprene

P01

Marek Jackowski

Department of General, Gastroenterological, and Oncological Surgery Collegium Medicum, Nicolaus Copernicus University, Toruń, Poland

Protocols for colorectal cancer biomarkers

Marek Jackowski (1), Jacek Szeliga (1), Tomasz Ligor (2)(3), Bogusław Buszewski (2)(3)

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(2) Department of Environmental Chemistry and Bioanalysis, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland
(3) Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Toruń, Poland

Background: Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world. In Europe, it is the second most common cause of cancer-related deaths. With volatolomics approaches, investigation of volatile profiles in breath and fecal samples related to CRC have been conducted. The main aim of the study was standardization
of methodology for recruitment strategy and clinical trials.
Methods: We have to find the basic parameters for collecting biological samples in many diseases of the large intestine, ranging from physiological conditions to inflammation and benign or neoplastic lesions. We also try to assess the normal background for the control group, and take into account the differences in inflammatory bowel diseases, colorectal cancer. Other important parameter is to establish inclusion and/or exclusion criteria for groups to have patient protocol equal for all centres.
Results: Histological type, tumor staging, grade and location were recorded for patients with previously diagnosed colorectral cancer. Additionally, CEA and Ca19.9 tumor markers in the blood were determined. The control group consists of persons which did not have any colorectal pathology in the colonoscopy within the last 6 months. Additional data such as age, gender, medications, comorbidities, smoking status were collected.
Conclusions: A fecal sampling protocol defining standard sampling conditions and a protocol for collecting breath samples from patients have been developed. The scheme of qualifying patients and protocol for collection biological samples were approved by the ethics committee.
Acknowledgements: This work was supported by The National Centre for Research and Development (Warsaw, Poland) "Airborne Biomarkers for Colorectal Cancer" project (ERA-NET TRANSCAN/023/2018).

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P02

Tomasz Ligor

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Extraction and identification of volatile organic compounds in fecal samples by SPME and dynamic headspace thermal extraction followed by GCMS

Tomasz Ligor (1)(2), Monika Śmiełowska (2), Fernanda Monedeiro (2), Jacek Szeliga (3), Marek Jackowski (3), Bogusław Buszewski (1)(2).

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Background: Various VOCs exist in breath, saliva, urine and sweat can be used for metabolomic study. Breath volatiles are frequently used for this purposes especially for searching of pulmonary diseases' markers. However volatiles emitted from feces can be useful for colon diseases especially colorectal cancer. Therefore VOCs in headspace of stool samples gives the opportunity to find colon cancer biomarkers. Such study can be useful for identification of specific volatiles related to colon diseases. Our work was focused on developing of extraction and analyses of fecal samples [1].
Methods: The analyses were performed by means of gas chromatography and mass spectrometry. Two different method were used for volatiles preconcetration over the samples. Firstly, solid phase microextraction (SPME) and then dynamic headspace thermal extraction and trapping on sorbent tubes followed by thermal desorption (TD) was used for sample preparation.
Results: The efficiency of SPME and micro‑chamber thermal extractor (μ-CTE) was compared. In this study, parameters that influence the VOCs extraction were evaluated. Fecal samples from healthy volunteers were analyzed. More than 100 volatiles were found. These are mainly short chain organic acids, volatile sulfur compounds (VSC), phenols, heterocyclic, terpenes and hydrocarbons. Mass spectrum libraries were used to identify of VOCs.
Conclusions: Micro‑chamber thermal extraction followed by thermal desorption and GCMS analysis allows for quick and efficient extraction of hundreds volatiles over the fecal samples. The main limiting factors are the presence of moisture in the stool samples which negatively affects the performance of the mass spectrometer analyses and chromatographic separation in the capillary column. However, headspace SPME GCMS can be regarded as a simply method with limited sensitivity.
Acknowledgements: This work was supported by The National Centre for Research and Development (Warsaw, Poland) "Airborne Biomarkers for Colorectal Cancer" project (ERA-NET TRANSCAN/023/2018).

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P03

Mahya Khaki & Matthias Friedrich

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The predictive value of the heart rate response to breathing maneuvers for significant coronary artery disease

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Background: Simple breathing maneuvers (BM) with hyperventilation (HV) and breath-holds (BH), coupled with Oxygenation-Sensitive Cardiovascular Magnetic Resonance (OS-CMR) imaging, can reflect coronary vascular function(1-4). Recently, we could show that the response of the heart rate (HR) itself may reflect heart disease. We aimed to assess the predictive value of the heart rate (HR) response to the BM for the presence of significant coronary artery disease (CAD).
Methods: We enrolled 56 patients with suspected CAD and 14 age-controlled healthy controls. The CAD pre-test probability was assessed using a validated risk score (5, 6). HR was recorded during 2-min normal breathing, followed by 1-min deep and paced (30 RR/min) HV and a subsequent end-expiratory maximal BH. We measured the BH-induced HR recovery (HRR-BH, %) relative to peak HR at HV (Fig. 1). Significant CAD was defined as an inducible perfusion deficit in stress CMR perfusion or, in patients undergoing invasive coronary angiography.
Results: Significant CAD was found in 39/56 patients (61±13 y, 46% female). Patients with CAD had a significantly lower HRR-BH (11.4%±6.5) than healthy controls (26.5±11.1%) and patients without stenosis (20.6±14.9%) (Fig. 2). An HRR-BH of ≥24% had a sensitivity of 97.4% and a negative predictive value of 85.7% (area under the curve 0.71), Fig. 3. In patients with intermediate (10%) and high (29%) pre-test probability of CAD, a normal HRR-BH (>24%) decreased the post-test probability to <3% (Fig. 4).
Conclusions: In patients with suspected coronary artery disease, the heart rate recovery following a simple breathing maneuver has a high negative predictive value for ruling out significant coronary artery disease. It may serve as a gatekeeper to improve patient selection for further diagnostic testing, specifically by correctly reclassifying patients with an intermediate or high pretest probability but a normal HR response from high-risk to low-risk.

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P04

Agapios Agapiou

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VOCs and PM in confined environments

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Background: Indoor air pollution in confined places has attracted the interest of scientists. Daily exposure to air pollutants, such as Volatile Organic Compounds (VOCs) and Particulate Matter (PM), is daily taking place, especially to confined premises and this can cause hazardous effects on the individual's health.
Methods: The sampling of VOCs was performed on Tenax sorbent tubes using 3 different analytical techniques (passive, active, and dynamic headspace (DH)), and analysed using thermal desorption coupled with gas chromatography/mass spectrometry (TD-GC/MS). Next to VOCs sampling, PM of different aerodynamic diameters 1, 2.5, 4, and 10 μm (PM1, PM2.5, PM4, PM10) were measured (portable system).
Results: A number of VOCs were detected in low ppbv levels, including BTEX, benzaldehyde, styrene, mesitylene, phenol, ethyl acetate, chlorinated compounds, siloxanes, etc. PM measurements revealed elevated values in the examined places.
Conclusions: Humans are exposed to various air pollutants (VOCs, PM) through different micro-environments, and therefore more actions need to be taken (e.g. better ventilation, use of greener products) to reduce human exposure.

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P05

Tanja Zivkovic Semren

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchatel, Switzerland

Workflow development for real-time exhaled breath analysis by secondary electrospray ionization coupled to high resolution mass spectrometry

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Background: Volatile organic compounds present in exhaled breath provide valuable information about biochemical processes occurring in the body. Real-time analysis of exhaled breath has gained traction in recent years and has the potential to become the method of choice for application in medical diagnosis and/or personalized medicine. Accurate interpretation of data obtained from exhaled breath analysis requires an understand of how confounding factors, such as beverage intake, can impact the composition of volatile organic compounds in exhaled breath.
Methods: We recruited volunteers to evaluate the influence of beverage intake on the volatile organic compounds present in exhaled breath. We performed real-time exhaled breath analysis using a commercially available secondary electrospray ionization source (Super SESITM) coupled to a Q Exactive HF mass spectrometer (MS). Volunteers were asked to consume a beverage of their choice from a pre-determined list of options and were subject to exhaled breath analysis to study kinetics at five different time points: before beverage intake and at 0, 10, 20, and 30 min after consumption.
Results: Our results showed that the Super SESITM system coupled to a Q Exactive HF MS provided reproducible compound profiles of exhaled breath. Statistical analyses confirmed the presence of numerous distinct compounds in exhaled breath after beverage intake. Our untargeted approach allowed us to observe the kinetics of these compounds over a 30-min run-time. The abundance of most compounds increased immediately after beverage consumption and decreased rapidly thereafter (i.e., after 10 min), exhibiting well-defined washout patterns in the respiratory tract during the 30-min monitoring period.
Conclusions: Although beverage consumption is primarily related to the gastrointestinal tract, compounds originating from various beverages can be detected in exhaled breath after consumption. Because of the increased levels of exogenous compounds present in exhaled breath following beverage intake, detection of endogenous metabolites may be partially suppressed. Based on our results, we conclude that a standardized approach for real-time exhaled breath analysis should require that participants avoid beverage intake for at least 30 min prior to sampling.

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P06

Rosaria Orlandi

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Secondary Electrospray Ionization– High Resolution Mass Spectrometry (SESI-HRMS) profiling of exhaled breath of head and neck cancer patients for clinical practice

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Background: Head and neck cancer squamous cell carcinomas (HNSCC) are tumors showing poor prognosis and a significant rise in incidence, largely due to the Human Papilloma Virus -related subtype. The increasing burden and the significant improvement in survival offered by early diagnosis highlight the urgent need of a non-invasive tool for a fast and reliable detection at onset and at relapse. Application of breath analysis to clinical practice has the potential to reshape the detection of HNSCC.
Methods: Exhaled breath was collected in sterilized nalophan bags and analyzed by SuperSESI (Fossiliontech) coupled with LTQ Orbitrap Elite (Thermo Fisher) using an untargeted approach. VOCs detection occurred in a few minutes without any sample pre-treatment. Pre-analytical and analytical steps were fully standardized by adherence to strict Standard Operating Procedures and a number of Quality Controls steps, following internationally recognised standards and practices. Data analysis integrated pre-processing methods for quality control and management of non-biological variability, based on our previous work [1].
Results: Exhaled breath of 169 head and neck cancer patients and 154 control subjects were profiled by SESI-HRMS. Information related to diseases, use of drugs and life-style of participants, along with clinical data of patients were linked to breath data. Data variability and influence of confounders such as gender, age and smoke were evaluated before to address unbiased pattern discovery.
Conclusions: Our study supports the feasibility of real time detection techniques and the importance of sample quality assessment and quality control of data for clinical applications of breath analysis. Moreover, it provides the necessary tools to carry out larger studies towards the development of clinically relevant diagnostic breath tests.

This work was supported by MIUR (5 x 1000 Funds – 2013) and by the Italian Ministry of Health (Ricerca Finalizzata 2016, RF-2016-02361759 and 5 x 1000 Funds – 2013).

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P07

Tara M. Lovestead

Applied Chemicals & Materials Division, NIST, Boulder, CO

A pilot study to determine if THC can be detected in breath aerosols collected from legal market cannabis users with an impaction filter device

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There are no reliable methods, either chemical or behavioral, for detecting impairment from cannabis use. Δ^9-tetrahydrocannabinol (THC-the primary psychoactive component of cannabis) can be detected in breath following exposure to cannabis and is a promising indicator for recent use.1-3 While alcohol breathalyzers are commonplace, THC is a very different compound than ethanol. THC is lipophilic, can be stored in the body, exhibits non-uniform elimination, and is non-volatile,4 requiring concentration from multiple exhalations. We hypothesize that it is carried in the breath aerosols. We conducted a pilot study in conjunction with an ongoing study of experienced cannabis users at the Univ. of Colorado to ascertain if THC concentrations in breath aerosols collected with an impaction filter device can differentiate abstinence from recent use.

Methods: Breath samples were collected from a subset of participants at a baseline assessment and at an experimental session conducted in a van before (pre-use) and ~ 1 h after naturalistic cannabis use (post-use). We obtained 35 breath samples from 14 participants. 6 breath samples (2 baseline, 2 pre-use, 2 post-use) were used for liquid chromatography with tandem mass spectrometry (LC-MS/MS) method development. For the remaining 29 samples, we improved our chromatography, sample preparation and calibration methods to quantify 5 cannabinoids and metabolites, if present.

Result: Overall, we found at least one measurable cannabinoid in 83% of post-use samples. THC was detected in 70% of the post-use samples and in 33% of abstinence (baseline and pre-use) samples. No difference in THC concentrations were found. We also observed 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) in 5 of the first 6 samples, but below the limit of quantitation.

Conclusions: To the best of our knowledge, this is the first-ever observation of THC-COOH in breath. It is unlikely that a breath measurement at a single timepoint will be sufficient to differentiate abstinence vs recent cannabis use for experienced users.

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P08

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Quartz-enhanced photoacoustic detection of ammonia in exhaled breath

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Background: Ammonia (NH_3) concentration exhaled from human alveoli is several hundred of parts per billion (ppb). Abnormal elevated (>1 part per million-ppm) levels are linked to kidney failure [1]. Also, a strong correlation between the level of respiratory NH3 and blood urea nitrogen in end-stage renal disease patients during hemodialysis is demonstrated [2]. Real-time measurement of in exhaled breath can be thus employed as a powerful tool to detect renal failure or to track hemodialysis process.
Methods: A sensor for exhaled NH_3 monitoring is presented [3], exploiting quartz-enhanced photoacoustic spectroscopy (QEPAS), which has been widely demonstrated as a highly sensitive and selective technique for trace gas sensing [4], including NH_3 [5]. An erbium-doped fiber amplifier targeting the 1531 nm NH_3 absorption line with 3 W optical power was employed as the excitation light. A custom spectrophone detected the photoacoustic signal.
Results: The sensor performance was first optimized in terms of spectrophone parameters, laser power and modulation. Linear responsivity was demonstrated to 10 - 0.2 ppm NH_3 concentrations in 1.5% humidified standard air. A minimum detectable concentration (MDC) of 14 ppb was obtained. Long-term stability was evaluated by Allan variance analysis.
The sensor was tested for breath analysis applications by continuously measuring NH_3 level exhaled by 3 healthy volunteers. Exhaled ammonia flowed into the sensor in real time by a custom gas sampler. With no breath flowing, the QEPAS signal decreased rapidly to the noise level, demonstrating the absence of memory effects. NH_3 levels of 170 ppb - 230 ppb were measured.
Conclusions: An NH_3 QEPAS sensor is presented, with an MDC of 14 ppb. The sensor was tested for real-time ammonia monitoring in exhaled human breath. Experimental results confirm the potentiality of the sensor for renal disease screening and hemodialysis treatment progress monitoring, whose exhaled NH_3 level is usually >1 ppm.

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P09

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Exhaled volatile organic compounds during inhaled mannitol challenges in adults

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Background: Inhaled mannitol provokes bronchoconstriction via mediators released during osmotic degranulation of inflammatory cells in asthma, and hence represents a useful diagnostic test and model for acute attacks, especially in phenotypes that share this mechanism such as exercise induced asthma. We hypothesised that mannitol challenge would trigger changes in exhaled volatile organic compounds (VOCs), generating novel insights into the origins of such VOCs and identifying potential biomarkers for future investigation.
Methods: Participants with a doctor-confirmed diagnosis of asthma, or suspected asthma being investigated by mannitol challenge were recruited. Inhaled mannitol challenges were performed, and in participants with hyperresponsiveness, a sham challenge was performed <14 days later. VOCs were collected before and after challenges and analysed using gas chromatography-mass spectrometry. Duplicate breath samples were taken for reproducibility assessment using interclass correlation coefficient (ICC). Pre-defined VOCs were extracted from samples using a semi-targeted approach including some reported previously from asthma. Univariate and multivariate (sparse Partial Least Squared Discriminant Analysis) analyses were carried out.
Results: Forty-six patients (mean [SD] age 52 [16] years) completed mannitol challenge and significant bronchoconstriction occurred in 16 (35%), 15 of whom attended for a sham challenge. Fifty-nine previously reported key asthma VOCs were identified, 9 were excluded due to poor reproducibility (ICC<0.6). Levels of 16 VOCs changed ($p<0.05$) following mannitol challenge, of which 12 also contributed to the multivariate model, with a classification error rate of 13.5% and area under the receiver operating characteristic curve of 0.90. Levels of 6 of these 16 VOCs also changed following the sham challenge, along with 3 further VOCs. In patients who had positive mannitol challenges who subsequently had a sham challenge, distinct VOCs signatures were observed following active and sham challenges.
Conclusion: Distinct changes in exhaled VOCs during mannitol and sham challenges were identified. Those differentially expressed VOCs merit further investigation as potential biomarkers of airway inflammation and bronchoconstriction in asthma.

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P10

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Pre-clinical exogenous volatile organic compounds (EVOCs) Probes screening and optimization for chronic liver diseases detection

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Background: The sole test approved for non-alcoholic steatohepatitis (NASH) diagnosis is liver biopsy, an invasive procedure that can lead to complications [1]. Surrogate methods lack adequate performance in early NASH stages. Breath measurement of exogenous volatile organic compounds (EVOC®) Probes represents an alternative approach for NASH detection and prognosis [2]. Here we report a pipeline for screening and identification of volatile organic compounds (VOCs) that have potential as EVOC Probes and are differentially metabolized by NASH hepatocytes in vitro.

Methods: Breath, from fasted healthy subjects, was collected before, and at different timepoints after oral administration of a formulation with high bioavailability for an EVOC Probe and analyzed using gas-chromatography mass-spectrometry (GC-MS) [3].

Organ on a chip human hepatocytes were treated to generate a model of healthy or NASH liver [4] and exposed to media containing the EVOC Probe. Headspace analysis of media collected at different timepoints was performed using GC-MS.

Results: Fasting resulted in undetectable breath levels of the EVOC Probe and its bioproduct. Ingestion of the EVOC Probe induced a spike of both compounds on breath after 20 minutes.

In hepatocyte-free controls, the EVOC Probe showed progressive decline due to spontaneous evaporation. The presence of hepatocytes induced a stronger EVOC Probe reduction, due to metabolic conversion. In the same samples, no bioproduct was detected in the hepatocyte-free control. In the presence of healthy hepatocytes, amount of bioproduct peaked 6 h after exposure and showed a reduction after 24 h, whereas, in the presence of NASH hepatocytes the bioproduct kept on increasing over 24 hours.

Conclusions: We have shown for the first time that NASH-induced metabolic alterations are detectable using an EVOC Probe safe for human consumption. These data indicate that breath analysis using EVOC Probes could be a suitable basis for a NASH detection test.

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P11

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Annotation of biomarkers in exhaled breath: combining real-time mass spectrometry and two-dimensional chromatography-mass spectrometry

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Background: Exhaled breath analysis by Proton Transfer Reaction - Mass Spectrometry (PTR-MS) allows the on-line, real-time detection of biomarkers of pathologies, but the understanding of the pathophysiological mechanisms implies the annotation of the biomarker candidates with a high confidence level. Following the discovery of candidate VOC biomarkers for COVID-19 [1], we implemented a combined PTR-MS and thermal desorption, two-dimensional chromatography coupled to mass spectrometry (TD-GCxGC-MS) analysis to enable their identification.
Methods: Breath samples from intensive care unit patients and analytical standards of the candidate biomarkers (as obtained from library search) were analysed with both i) PTR-MS real-time analysis (PTR-Qi-TOF with liquid calibration unit, Innsbruck, Austria) and ii) TD-GCxGC-MS (BT4D, Leco, USA) from clinical samples collected on Tenax TA tubes (Markes, United Kingdom) or tubes spiked with the analytical standards. PTR-MS data analysis was performed with the ptairMS R-package.
Results: Twenty analytical standards corresponding to 8 different m/z candidates (PTR-MS $[M+H]^+$) were analysed. Signals were recorded at the expected m/z for PTR-MS for 15 standards. GCxGC-MS analysis allowed the detection of all compounds, adding the chromatographic separation of isomers and confirmation of VOC identification with comparison to NIST mass spectra library.
The comparison of analytical standards and clinical samples was performed and allowed the level 1 annotation of 1 candidate biomarkers.
Conclusions: Combining real-time and chromatographic analysis of breath samples may be useful for the rapid detection and the annotation of VOC candidate biomarkers, highlighting the interests of breath analysis for clinical use and pathophysiological studies.
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P12

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Investigation of an individual with low exhaled isoprene

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Due to its relative high abundance and variable nature, isoprene (2-methyl-1,3-butadiene) is one of the exhaled compounds most often evaluated for biomarker discovery purposes. As a result, identification and investigation of individuals with background levels of endogenous isoprene is of great interest. Here, exhaled breath (EB) from an individual found to have background levels of isoprene was evaluated. Experiments were performed with this individual at rest; 2) during exercise and 3) from close family members [1-2].

EB was analyzed by both, proton transfer reaction mass spectrometry (PTR-MS, Ionicon Analytik), and thermal desorption gas chromatography mass spectrometry (TD-GC-MS, Markes International, ThermoFisher Scientific). Both instruments were externally calibrated for isoprene and acetone using a 1ppm standard gas canister. Breath samples consisted of lower airway exhalations, except for those collected before, during, and after a 15-minute exercise on an exercise bike (whole breath). All data were evaluated for both isoprene and acetone concentrations using either the PTR Viewer or Tracefinder EFS software packages.

Data from both the analyses confirm the identified individual has background exhaled isoprene quantities (PTR-MS: 8.99ppb, TD-GC-MS: <1.81ppb) when compared to eight control individuals (PTR-MS: μ=196.2±45.7ppb, TD-GC-MS: μ=136.8±45.7ppb). Furthermore, the results suggest the identified individual lacks the hypothesized muscular stores of isoprene illustrated by a 1.22% reduction in isoprene upon exercise initiation [1]. Whereas eight control participants show a 36.8%±10.7 increase in isoprene after exercise start. EB collected remotely for TD-GC-MS analysis from the individual's family, grandmother, father, mother, and sister, suggests the low levels of isoprene (TD-GC-MS: 28.5-77.2ppb) are observed from the parents and grandmother while "normal" levels are found in the sibling (182.0ppb). These results suggest a genetically recessive gene is likely responsible for the isoprene phenotype observed [2].

These data validate identification of an individual with background levels of exhaled isoprene while also probing for potential mechanism. The results suggest that the individual may have a recessive gene accounting for the observed isoprene levels.

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P13

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Alveolar nitric oxide in COPD – a 2-year follow-up

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Background: Alveolar nitric oxide (C_ANO), also referred as NO from the gas exchange area, has been described to be increased in chronic obstructive pulmonary disease (COPD) [1]. There are few studies with long-time follow-up and these studies include treatment effects of corticosteroids after one week [2] and after four weeks [3]. Both studies showed no difference in C_ANO between the visits. Our aim was to investigate the stability of C_ANO over a 2-year period in a cohort of COPD subjects recruited from the Swedish Tools for Identifying Exacerbations (TIE-study).
Methods: A total of 110 subjects (45 males), age 68±8 years, 19% current smoker, with spirometry-verified COPD diagnosis were included. Disease severity was classified at the inclusion visit according to GOLD 2021 groups A/B/C/D: 40%, 39%, 6% and 15%, respectively. Subjects were clinically stable at inclusion, and during the 1 and 2 year followup. Data were collected through questionnaires including the dyspnoea scale (mMRC) and measurements of lung function. C_ANO was estimated with the non-linear method of Högman-Meriläinen Algorithm (EcoMedics, CDL88)1.
Results: During the 2-year follow up C_ANO increased from 1.3 (0.6, 2.1) to 1.7 (1.1, 2.3) ppb, median (Q1,Q3), p=0.02. A decline in lung forced vital capacity was seen (p≤0.001), without alteration in F_ENO_{50} between the visits. At inclusion 42% reported dyspnoea (mMRC ≥2), after 2 years this was 44%. The subjects who increased their dyspnoea score by 1-3 points (n=32) over the 2 years also had an increase in CANO from 0.9 (0.5, 2.1) ppb to 1.8 (1.1, 2.3) ppb, p=0.02. The subjects with dyspnoea score -1 point or the same value (n=78) had no increase in CANO (1.4 (0.7, 2.1) ppb resp. 1.4 (1.1, 2.3) ppb. When grouping C_ANO into low, medium, and high, see table 1, we found a shift towards higher values from baseline to 2-year follow-up (p=0.02).
Conclusion: During the 2-year follow-up alveolar NO slightly increased. Our hypothesis is that an increase in C_ANO is related to remodelling of the gas exchange area, thus affecting the subjects' perception of dyspnoea.

Table 1. Number of subjects divided into CANO groups low, medium, and high.

C_ANO groups	Baseline	1-year follow-up	2-year follow-up
C_ANO <1 ppb	46 (42%)	27 (24%)	23 (21%)
C_ANO 1-2 ppb	33 (30%)	48 (44%)	49 (44%)
C_ANO >2 ppb	31 (28%)	35 (32%)	38 (35%)

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P14

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Selective monitoring of breath isoprene by a sensor during exercise and at rest

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Isoprene has received widespread attention in breath research because of its potential to serve as a sensitive and non-invasive biomarker for the detection and monitoring of several metabolic effects. To date, research activity on breath isoprene focused on mass spectrometry-based measurement techniques, which are not portable and require skilled operators. Here, we show, for the first time to our knowledge, selective isoprene monitoring in exhaled breath (148 breath samples, 60–1250 parts-per-billion, ppb) with an inexpensive, user-friendly and compact filter–sensor device[van den Broek, 2022]. This detector is based on a previously developed concept comprising a sorption filter of activated alumina that removes hydrophilic volatiles ahead of a micro gas sensor consisting of chemoresistive Si-doped WO3 nanoparticles to quantify the isoprene down to few ppb concentrations. When tested on humans during exercise and at rest, the detector accurately followed breath isoprene dynamics in linear (Pearson's coefficient 0.89) correlation to proton transfer reaction mass spectrometry measurements. Most importantly, the output from the device is not interfered by high and variable concentrations of other breath volatile compounds, specifically acetone, ethanol and methanol. This isoprene detector can be readily applied for online monitoring of physical activity.

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P15

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Monitoring rapid metabolic changes in health and type-1 diabetes with breath acetone sensors

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Routine detection of health parameters is desirable to recognize the early onset of metabolic diseases (e.g., diabetes mellitus) and to personalize their treatment. Promising are non-invasive, affordable and portable technologies, such as breath sensors. Yet, the selective monitoring of breath markers (e.g., acetone for lipolysis) with sensors to track metabolic changes that can reveal disease-related abnormalities remains challenging. Here, subtle breath acetone changes during fasting, exercise and glucose ingestion are tracked in two model situations: Patients suffering from type-1 diabetes mellitus (T1DM) and healthy subjects (total: 19 volunteers) were monitored using chemoresistive sensors based on Si/WO3 nanoparticles [Weber, submitted]. Specifically, each subject cycled after overnight fasting to stimulate fatty acid oxidation followed by an oral glucose tolerance test (OGTT), as monitored by capillary blood glucose and β-hydroxybutyrate (BOHB) concentrations. The sensor recognized accurately the individual breath acetone patterns before and after OGTT (both R2 = 0.9) at negligible interference, for instance, from glucose ingestion-associated volatiles (e.g., ethanol) or isoprene, as confirmed by high-resolution mass spectrometry.

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P16

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Data visualization for real time mass spec-based breath analysis in clinical setups

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Background: Mass spectrometry is an indispensable research tool for basic research and laboratory medicine. New instruments with better mass and time resolution produce more accurate data than ever before. The throughput is much higher and by new setups – often in real time – produced data sets are getting larger and larger. Quick and efficient interpretation of the data remains a great challenge since hundreds of compounds can be detected which makes it hard to identify the relevant substances. There is a need for improvement and standardization of data analysis, especially regarding first view and data evaluation.

Methods & Results: Therefore, we developed a tool with a graphical user interface, that performs automated statistical analysis and visualizes the data using box plots including labels of statistical significance on first sight. With this tool an easy comparison of sample types (e.g., two different patient groups) and/or changes of concentrations over a defined time period is possible. The boxplots can be enhanced by violin-plots and normalization of data on a chosen time point is possible. In contrast to other visualization techniques such as heatmaps, this tool includes fast and simple automated analysis for visualization of statistical significance. The tool is open source and based on the free coding language R. Therefore, adaptations to different research questions (e.g., statistical test or axis labeling) and implementation of additional statistical methods can easily be realized.

Conclusions: This new algorithm represents an innovative and effective tool for first data view in the field of bioanalytics. Perspectively, this kind of algorithm delivers visualization of data, making a quick preselection of potential biomarkers possible.

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P17

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Online SESI-HRMS breath analysis after a nutritional intervention challenge

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Background: Online breath analysis using secondary electrospray ionization coupled to high-resolution mass spectrometry (SESI-HRMS) can be used as a sensitive method for metabolomics and biomarker discovery. [1] The analytical characteristics of this technique make it ideal for medical diagnostics and nutrition research.
Methods: Eleven subjects underwent a nutritional intervention on three separate days, consisting of a shake with all macronutrients (proteins, carbohydrates, and lipids) balanced. [2] Exhaled breath was measured once before the intervention (baseline measurement) and up to six hours afterwards, with thirty-minute intervals. Breath samples were analyzed using a SuperSESI ion source (Fossil Ion Technology, Spain) coupled to an Orbitrap Q-Exactive mass spectrometer (Thermo Fischer Scientific, Germany). After spectra preprocessing, ANOVA-simultaneous component analysis (ASCA) estimated the variation stemming from the individual experimental parameters (subject, day, time). Compound annotation of a subset of intervention-related features was performed through online (collision-induced dissociation) CID experiments.
Results: ASCA analysis revealed that the major contribution towards variation stems from the individual differences, being responsible for 30 % of the observed variation. This hinted at a considerable inter-individual variability compared to the day-to-day variation (intra-individual variation).
Pathway analysis using the mummichog algorithm [3] highlighted pathways mostly associated with the metabolism of linoleate, butanoate, as well as amino sugars.
Additional compound annotation was performed with online CID experiments. The identified compounds mainly fell into three major categories: fatty acids, amino acids, and hydrocarbons.
Our findings were compared with previously reported time traces [2] for such a nutritional intervention, and the median time traces of the detected features were K-means clustered. Our breath data detected four cluster trends, indicating that online SESI-HRMS can follow Nutri-triggered metabolomics responses.
Conclusions: Online SESI-HRMS was shown to be a valid methodology for following the effect of a nutritional intervention on the human breath metabolome.

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P18

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Development and testing of a vapor generator for quantification of exhaled breath metabolites

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Background: The calibration of molecular sensors is of major importance, especially for quantitative measurements. Quantification of exhaled breath metabolites is associated with measuring the device's analytical performance, accuracy, reproducibility, reliability, and stability. This study reports the development, testing, and analytical evaluation of a vapor generator capable of producing gas standards (either single or multi-component) in both periodic and dynamic ways for use in breath metabolomics investigations.

Methods: Quantification of selected exhaled breath metabolites was done using a built-in-house vapor generator based on the controlled evaporation of volatile or semi-volatile chemical analytes and their diffusion into a carrier gas stream. The main components of our vapor generator are a) a mixing chamber, b) three individual temperature-controlled evaporation chambers, in which liquid analytes are introduced through a side injection port, c) four mass flow controllers, d) an automation platform controlled by software. During our experiments, the principal sensor for the characterization of the developed system was an online secondary electrospray ionization (SESI) source coupled to a high-resolution mass spectrometry (HRMS) system. SESI-HRMS is a powerful, well-established, and robust analytical technique ideal for in-depth breath metabolomics characterization offering high sensitivity, fast and accurate analysis [1]. This vapor generator was benchmarked against a commercial gas generator based on permeation tube technology [2].

Results: Experiments were undertaken for volatile short-chain fatty acids (SCFA) at different concentrations and flow rates. Both individual compounds and mixtures were tested. Gas-phase experiments were performed at concentration levels from low ppt to low ppm and various relative humidity levels. The experimental results obtained showed a precise and repeatable production of gas standards with excellent linearity within the examined concentration range, low ppt detection limits, and fast response times.

Conclusions: The developed vapor generator has the capability of producing periodic and dynamic pulses of gaseous standards of interest in a precise, controllable and reproducible way.

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P19

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Exhaled breath condensate profiles of US Navy divers following prolonged hyperbaric oxygen (HBO) and nitrogen-oxygen (Nitrox) chamber exposures

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Background: In military diving operations, breathing gas mixtures ustilizing high partial pressures of O_2 are used to reduce decompression obligation from prolonged dives, as well as for the treatment of decompression illness. In addition to treating decompression sickness, the Undersea and Hyperbaric Medical Society recognizes the use of hyperbaric O_2 (HBO) as an accepted indication for 13 other medical conditions [1]. However, breathing high concentrations of O_2 for prolonged periods of time results in pulmonary oxidative stress that can reduce lung function and lead to pulmonary O_2 toxicity (PO_{2tox}). Currently, detection of PO_{2tox} relies on symptomology and/or spirometry measures of pulmonary function. In this study, we aim to determine if there is a specific breath profile of compounds in exhaled breath condensate (EBC) that is indicative of the early stages of pulmonary hyperoxic stress/ PO_{2tox}.

Methods: In a double-blind randomized crossover study, 14 male US Navy divers conducted a nitrox (30.6% O_2 balance N_2) and a HBO (100% O2) 390 min hyperbaric chamber dive at 2 ATA separated by 1 week. EBC samples were taken immediately before and after each dive and subsequently underwent a targeted and untargeted metabolomics analysis using liquid chromatography coupled to mass spectrometry (LC-MS).

Results: Multivariate data analysis of the identified metabolites in EBC showed clear differences in the pattern of results between the nitrox and HBO dives, as well as discrimination between EBC samples collected before and after each dive. A Partial Least-Squares Discriminant Analysis (PLS-DA) of the normalized (relative to predive) untargeted data (excluding one outlier) gave good classification abilities with an area under the curve of 0.99 (± 2%) and sensitivity and specificity of 0.93 (± 10%) and 0.94 (± 10%), respectively. The resulting classifications allowed the characterization of specific biomarkers, such as fatty acyls, that may explain metabolomic changes resulting from prolonged HBO exposure or decompression stress.

Conclusions: These promising results allowed for the identification of breath biomarkers in EBC that are differentially related to either pulmonary hyperoxic stress or decompression stress.

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P20

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Effects of a volatile compound filter on breath profiles measured by online high-resolution mass spectrometry

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Background: Inhaled volatile organic compounds (VOCs) originating from the environment may influence exhaled breath profiles. Such potential effects have not been assessed systematically for online breath analysis to date.
Methods: Repeated breath samples of 24 adult volunteers were collected with and without an activated carbon air filter for VOCs (Dräger X-plore Rd40 Filter 940 A2). The measurements were performed on an AB SCIEX TripleTOF 5600+ HRMS (m/z range 50 - 500, mass accuracy < 2 ppm) coupled to a SUPER SESI ion source. Within-subjects analysis was performed to assess the changes in the exhaled breath profiles after filter usage. Bland-Altman analysis was applied on known human breath markers and environmental contaminants.
Results: A total of 2059 m/z-features were recorded from exhaled breath samples of which 1417 differed significantly between measurements with and without the VOC-filter (adj. p-value < 0.05). On average, a relatively small bias was found when using the filter towards higher intensities. Bland-Altman analysis showed that the environmental contaminants such as different polysiloxanes, acetonitrile and methanol were found to be lower in intensities after filter usage. In contrast, some of the known human breath markers such as acetone, lysine and aldehydes experienced a small raise in intensity after filter usage.
Conclusions: Preliminary results indicate that VOC filters may partially suppress intensities of the environmental contaminants and increase the intensities of the markers associated with human metabolism. However, the effect was relatively small and not consistent over the whole breath profile. Therefore the additional complexity of using inspiratory filters in studies including younger children has to be considered.

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P21

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Diagnosis of SARS-CoV-2 infection from exhaled breath volatiles using GC-MS

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Background: The ongoing COVID-19 pandemic reveals the global need for alternative diagnostic tests for respiratory diseases. It is known that exhaled breath contains dozens to hundreds of volatile metabolites, and this volatilome profile can shift due to injury or disease. In this work, we examine the potential for exhaled breath vapor to serve as a diagnostic biospecimen for SARS-CoV-2 diagnostics.
Methods: Breath vapor samples were collected from symptomatic and asymptotic adults with confirmed COVID-19 disease, alongside symptomatic and asymptomatic controls. Collection occurred at the UC Davis Medical Center and from the surrounding Sacramento community under an IRB-approved protocol, #1636182. Demographic information about volunteers was collected through a questionnaire and review of electronic medical records. Breath was collected in Tedlar bags, then extracted onto Tenax TA sorbent tubes. A paired background/environmental air sample was collected for each breath sample. Samples underwent thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) analysis. GC-MS data were deconvoluted and aligned. Background VOCs were subtracted from breath samples. The dataset was randomly split into a training and validation set for machine learning algorithms to identify the COVID-19 breath signature.
Results, Conclusion: At time of abstract submission, sample collection and chemical analysis are ongoing. During the IABR Meeting, the authors will present the latest findings, detailing the accuracy, sensitivity and specificty of a breath-based GC-MS assay to diagnose COVID-19. We will present findings on whether COVID-19 biomarkers correlated with demographic factors such as age, ethnicity, or correlated with disease severeness and symptoms.

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P22

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The effect of sleeve gastrectomy on halitosis

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Objectives: The aim of this study was to investigate the effect of LSG on halitosis. In order to achieve this aim the following objectives were considered: (1) To evaluate the relationship between LSG and intra-oral halitosis by assessing the effect of LSG on the levels of volatile sulfur compounds (VSC) in breath, and halitosis-related bacteria. (2) To evaluate the relationship between LSG and extra-oral halitosis by assessing the effect of LSG on the levels exhaled acetone, and concentration of 3-hydroxybutyrate in the blood.

Materials and Methods: A Prospective longitudinal cohort design was used in this stud. The study subjects were recruited from patients' waiting list scheduled to perform LSG with a body mass index (BMI) of 35–50 kg/m^2. All included subjects were examined before LSG procedure and followed 1, 3 and 6 months after the procedure.

Part I (intra-oral halitosis): Clinical periodontal measurements including Plaque index (PI), gingival index (GI), and probing depth (PD) were taken at baseline, 1, 3, and 6 months post-surgery. Also, subgingival plaque samples were collected from each patient at each study interval. Quantification of the periodontopathogenic bacteria: *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *Fusobacterium nucleatum* (*F. nucleatum*), was performed using real time quantitative polymerase chain reaction (qPCR). In addition, breath samples were collected in order to analyze the concentration of hydrogen sulfide (H2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3) using portable gas chromatography (Oral Chroma™).

Part II (extra-oral halitosis): Breath samples were collected with a portable breath ketone analyzer for measurement of acetone concentrations, and blood samples were taken for measurement of 3-hydroxybutyrate concentrations. Both breath, and blood samples were taken at baseline, 1 month, 3 months, and 6 months post-surgery.

Results: Initially, 43 patients were included in the study, 39 patients completed the study. The study subjects were 12 males, and 27 females with age mean (±SD) of 32.2 (±10.4).

Part I (intra-oral halitosis): Repeated measurements one way ANOVA showed a significant increase of PI, and GI after one month of surgery with a mean (1.3, 1.59) respectively P-value < 0.001 compared to baseline. No significant changes were found in PD throughout the study. The levels of hydrogen sulfide (H_2S), and methyl mercaptan (CH_3SH) have shown statistically significant increase during the first month after the surgery (P = 0.02, 0.01) respectively. Also, the amount of P. gingivalis has increased at 1 month followed by a decrease at 3 and 6 months (P=0.004). F. nucleatum values elevated significantly through from baseline for the study period (P=0.003). No significant changes were found for T.forsythia, and T.denticola. Finally, P.gingivalis showed significant positive correlation with GI.

Part II (extra-oral halitosis): The mean concentration of breath acetone was 4.1, 3.4, and 3.8 parts per million (ppm) at 1 month, 3 months, and 6 months, respectively. There were no statistically significant differences between the postsurgical levels of breath acetone (P = 0.1). There was a statistically

significant increase in breath acetone at 1 month in patients with a high rate of BMI loss (≥5Kg/m2). At 1 month, the mean blood concentration of 3-hydroxybutyrate was higher in patients with a high rate of BMI loss than in those with a low rate of BMI loss (1.9 vs. 1.2 mmol/L; P = 0.049). The concentrations of breath acetone and blood 3-hydroxybutyrate were significantly correlated at the 1st month only.
Conclusions: This study demonstrates that LSG has an impact on both intra/extra-oral halitosis. This effect was great during the first month after the surgery followed by further decline at 3, and 6 months of follow-up. LSG was associated with Intra-oral halitosis due to the increase of VSC in breath which was accompanied by an increase in GI, PI, and P.gingivalis in the 1st month post-surgery. Also, there was a significant increase of acetone in the breath at 1 month after the surgery only in the group of patients with rapid weight loss. It is evident that LSG was associated with dental side effects that led to intra oral halitosis, and metabolic changes due to rapid weight loss that contributed to extra-oral halitosis. Therefore, it is recommended that dental health provider to be included as a member of the multidisciplinary team for the management of bariatric surgery patients to monitor their dental and periodontal condition during the entire process especially during the first month post-surgery.

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P23

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A core breath profile of healthy non-human primates

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Background: Non-human primate models are useful for studying human diseases1-4. To minimize harm to these sentient animals, their use is limited, and non-invasive approaches are favoured5-7. Breath collection is a relatively non-invasive, useful tool for studying animal health8-11. As such, we aimed to define a baseline breath volatilome of non-human primates which may inform future disease-related studies in these animals.
Methods: Sampling: 49 breath samples were collected, in 5 L Tedlar bags, from 30 healthy cynomolgus macaques at the University of Pittsburgh. They were then pumped onto 3-bed sorbent packed thermal desorption tubes which were then sealed and stored at 4 ˚C until analysis.
Sample analysis: Samples were analysed via thermal desorption (GERSTEL) coupled to GC×GC-ToFMS (LECO).
Data analysis: Data processing was done using Statistical Compare in ChromaToF (LECO). S/N thresholds were set, putative identification was carried out using the NIST 2011 library, and peaks were aligned. Once identified, the mass spectra of core features (found in 100% of samples) were visually inspected to assess the quality of the alignment and our confidence in the assigned IDs.
Statistical analysis: Artifact removal, data normalization and transformation were done using R. 30 samples were used for training and 19 (replicates) for validation. Through an FOO count we calculated the number of constituents in the core (found in 100% of samples), accessory (>10%, <100%) and rare (<10%) breath volatilome.
Results: 2,017 features total were found of which 125 comprised the core, 1,426 the accessory and 466 the rare. From the core, marked by aliphatic hydrocarbons, aromatics and carbonyls, we defined a useful criitcal core of 23 high abundance, low variance compounds.
Conclusions: This is the first step towards creating a list of core breath features in non-human primates. In future, we aim to further refine our methodologies that this work will serve to guide other breath studies associated with these animals.

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P24

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Analysis of urinary volatile organic compounds by electronic nose and GC/MS for prostate cancer diagnosis

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Background: Prostate cancer (PCa) is one of the most common cancers diagnosed among males worldwide. Current diagnostic methods for PCa are invasive and lack specificity. Serum prostate-specific antigen (PSA) has been considered the most important biomarker for PCa detection, but its use remains controversial. The diagnosis of prostate cancer is currently still a great challenge leading to the search for alternative non-invasive tests based on urinary biomarkers detected by biosensing devices [1,2]. An emerging promising approach is also devoted to the analysis of urinary Volatile organic compounds (VOCs) that may indicate the physiological and metabolic status of the individual and represent cancer biology [3].
The objective of this study was to identify urinary VOCs that may be sensitive and specific biomarkers for PCa, and develop an Electronic Nose as stand-alone instrument for potentially offering a clinically applicable noninvasive and rapid diagnostic method.
Methods: In this work we designed and built a device based on an array of chemoresistive gas sensors (Electronic Nose). The prototype of Electronic Nose, which has been named SPYROX, is able to analyze the response of the sensor array to the VOC complex of any (bio) liquid or solid sample, collected in the headspace of a suitable vial with gas-tight septum. The device also has the functionality of analyzing gaseous samples collected in appropriate bags for gas sampling, thus also allowing the possibility of analyzing the breath.
This activity was carried out in conjunction with the use of standard gas chromatography technologies and solid phase microextraction (SPME-GC/MS).
Results: A total of 71 urine samples were collected from a cohort of subjects, including 25 from control subjects and 46 from subjects with PCa prostate cancer.
A pattern of 27 VOCs with statistically significance for the discrimination between controls and PCa subject was identified. The data resulting from the statistical analysis of the sensor responses to urinary Volatile Organic Compounds (VOCs) and of the VOC patterns identified by the SPME-GC/MS analysis were compared and the possibility of developing a non-invasive diagnostic test for the prostate cancer (PCa) based on urinalysis evaluated as the result of the study.
Conclusions: The urinary VOC-based models based both on enose and SPME-GC/MS are promising alternative strategies for developing novel noninvasive and reliable method for PCa diagnosis.

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P25

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Metabolic phenotyping of acquired ampicillin resistance using microbial volatiles from *Escherichia coli* cultures

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Background: Antimicrobial resistance (AMR) represents a major global health concern. There is an urgent need to develop novel methods for the detection of AMR for implementation into routine clinical diagnostics. E. coli are a major cause of nosocomial infections including ventilator-associated pneumonia. Bacteria display species-specific profiles of volatile organic compounds (VOCs) which may be used as markers of pathogen infection. VOC profiling is a growing area of interest in clinical translation studies as VOCs are representative of metabolic phenotype. Recent studies have demonstrated differences in VOC profiles of susceptible and resistant bacteria [1]–[3]. Using a metabolomics-based approach, we sought to examine the differential volatile profiles of ampicillin-susceptible and -resistant E. coli.
Methods: Headspace analysis of ampicillin-resistant and -susceptible E. coli cultures was undertaken using thermal desorption-gas chromatography-mass spectrometry. Cultures were incubated for 6 h in drug-free, low concentration and high concentration ampicillin. Multivariate statistical analysis utilising unsupervised machine learning methods was undertaken to assess differences in VOC profiles.
Results: More than 30 compounds were differentially expressed in all conditions. Principal component analysis showed distinct separation of the resistant and susceptible strains. Hierarchical clustering analysis indicated that resistant bacteria were distinguishable from their susceptible counterparts using as few as six compounds.
Conclusion: We found that ampicillin resistant E. coli cultures exhibit altered VOC profiles compared to their susceptible counterparts both with and without antibiotic stress. This suggests fundamental differences in the metabolisms of ampicillin-resistant and -susceptible E. coli which may be detected by means of VOC analysis. This shows promise for the detection of resistant organisms using VOC analysis, with potential application in breath analysis.

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P26

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Breath biopsy OMNI: Advanced global breath VOC analysis

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Background: We developed Breath Biopsy® OMNI with the aim of providing reproducible, standardized data for the global analysis of volatile organic compounds (VOCs) on breath. Our aim is to support advancement in the breath research field by facilitating the identification and development of valid, clinically-relevant biomarkers. This work highlights the importance of distinguishing on-breath VOCs that arise within the body from VOCs that are simply present within breath. We also advocate for the wider use of breath or blank studies as a means to quantify improvements in methodology relevant to the detection of on-breath VOCs.

Methods: Breath Biopsy OMNI involves collecting breath samples using ReCIVA® Breath Sampler, controlling background signals using CASPER® Portable Air Supply and performing chemical analysis using high resolution accurate mass (HRAM) gas chromatography mass spectrometry (GC-MS). By making changes to the parameters throughout this process and measuring their effects we have found ways to improve performance.

Results: We define on-breath VOCs as those more than three standard deviations above the average background signal. In a study of 57 samples from four volunteers, our resulting method shows the capacity to detect a median of 517 on-breath VOCs per sample with median intrasubject RSDs of 26-36%. We have also applied the method to our work on chronic liver diseases and performed a global analysis of breath samples from 46 cirrhosis patients and 42 healthy volunteers. Our results reveal 29 VOCs of interest including several previously identified. We also show that several of these correlate with blood biomarkers of liver disease.

Conclusions: By performing a detailed review of our breath collection and analysis pipeline we have been able to make changes to improve our ability to reliably detect on-breath VOCs. In addition, we have demonstrated the value of BoB studies as a means to compare the performance of analytical methods.

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P27

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Detection of SARS-CoV-2 Omicron infection in exhaled breath from out-patients with mild respiratory symptoms

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Background: SARS-CoV-2 Omicron variant infection in minimally symptomatic individuals poses a diagnostic challenge due to the circulation of other respiratory viruses causing similar symptoms. To determine if a SARS-CoV-2 infection could be accurately diagnosed via exhaled breath samples in mildly symptomatic patients predominantly infected with Omicron variants, we compared the breath profiles of mildly symptomatic RT-PCR COVID positive individuals with healthy controls in an out-patient setting.

Methods: COVID-19 infected participants were recruited from an out-patient COVID-19 testing site. Sex and age matched healthy controls were recruited from a research facility. 3L of breath was collected from each individual into a Tedlar bag and immediately transferred to thermal desorption tubes using an Elf pump. Samples were analyzed via TD-GC-ToF-MS, and instrument performance was monitored using 104 parameters from 26 VOC's in a standard solution. Multivariance analysis by orthogonal partial least squares (OPLS-DA) was used to select predictive markers which were then further analysed using non-supervised principal component analysis (PCA-X) with 7-fold cross validation.

Table 1. Demographics of enrolled adult population

	All (%)	Positive (%)	Healthy Controls (%)
Subjects	38	19	19
Demographics			
Age years	39.97 ± 14.5	39.00 ± 14.5	40.95 ± 14.8
Male	15/38 (39)	7/19 (37)	8/19 (42)
Caucasian	22/38 (58)	12/19 (63)	10/19 (53)
Cigarette Smoke	5/38 (13)	4/19 (21)	1/19 (5)
Cannabis	8/38 (21)	7/19 (37)	1/19 (5)
Vape	4/38 (11)	3/19 (16)	1/19 (5)
Comorbidities			
Cardiopulmonary or Endocrine disorder	8/38 (21)	3/19 (16)	5/19 (26)
Immunocompromised	0/38 (0)	0/19 (0)	0/19 (0)

Results: Nineteen participants with RT-PCR confirmed SARS-CoV-2 Omicron infection and 19 healthy controls were enrolled into the study (table 1). The top three upregulated and four downregulated features with the furthest vector distance (p corr) from the centre of the OPLS-DA model were selected for further analysis with PCA-X. These candidate markers consisted of methylated hydrocarbons, ketones and cyclic compounds. The AUROC curve based on the 1st PCA-X component, gave an AUROC of 0.99 and was able to distinguish Covid-19 from healthy controls with high reliability and predictability of the PCA-X model (RX2=0.868 and Q2=0.64). These molecular features are being compared with other SARS-CoV-2 variants.

Conclusions: This study demonstrates that individuals with mildly symptomatic Omicron SARS-CoV-2 infection can be detected using exhaled breath analysis.

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P28

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Mass spectrometry profiling of exhaled breath of smokers to identify a signature related to tobacco use

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Background: Cigarette smoking is an important risk factor for inflammatory and oncologic diseases. Smoking habits directly and indirectly alter the breath composition, therefore breath analysis represents a great opportunity to reshape clinical diagnostics in smoke-related diseases. Additionally, smoke-related volatiles can be a confounding factor for breath analysis discovery studies of other diseases.
Methods: Forty-five healty male subjects (22 smokers and 23 non-smokers) were recruited and their breathprints were compared. All subjects performed spirometry, FeNO and exhaled CO tests, and their exhaled breath was collected and analyzed with Secondary ElectroSpray Ionization High Resolution Mass Spectrometry (SESI-HRMS). Subjects were asked to fast and refrain from smoking in the 2 hours before the first sampling. Smokers were allowed to smoke a cigarette and after 20 minutes all subjects' breath was sampled again. Data were extracted with MZmine software, pre-processed and analyzed following our R-based dedicated procedure [1].
Results: The measured CO concentration values in exhaled breath confirmed the smoking status and the number of smoked cigarette declared by the subjects. FeNO values were lower in the smokers group, in agreement with Högman findings [2]. Supervised analysis highlighted the presence of 2 signatures related to chronic and immediate (during study sampling) tobacco consumption.
Conclusions: This study proved the suitability of the SESI-MS analytical platform in the detection of signatures related to smoking habits. Further investigations with larger cohorts of smokers are aimed at validating the signatures, identifying the tobacco-related breath features and developing tobacco-related profiles predictive of respiratory diseases.
This work was supported by MIUR (5 x 1000 Funds – 2013) and by the Italian Ministry of Health (Ricerca Finalizzata 2016, RF-2016-02361759 and 5 x 1000 Funds – 2013).

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P29

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Headspace analysis of asbestos exposed mesothelioma cell lines

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Background: Malignant pleural mesothelioma (MPM) represents a significant diagnostic and therapeutic challenge and is almost always a fatal disease. MPM is associated with occupational exposure to asbestos mineral fibres, and despite a long the latency period of (up to 70 years), MPM is usually diagnosed at an advanced stage with limited treatment options. A non-invasive breath test has the potential to identify high risk individuals and possibly to diagnose MPM at an earlier and more treatable stage. For this reason, this study aims to fully characterise the headspace VOC profile of asbestos exposed mesothelial cell lines.

Methods: VOC analysis of headspace gas in cell culture flasks was performed as previously described [1] via SPME/GCMS analysis. MSTO-211H, NCI-H28 and MET-5a cell lines were exposed to 5µg/mL asbestos mineral fibres (Chrysotile, Actinolite, Amosite and Crocidolite, and Wollastonite as a non-asbestos fibre control) kindly donated by Santia Asbestos Management Ltd. Principle component analysis (PCA) was carried out using SiMCA Multivariate Analysis software (Sartorius, Göttingen), and specific VOCs were identified using the MetaboAnalyst 5.0 T-test and heatmap function [2].

Results: Principle component analysis shows distinct grouping of asbestos exposed and non-exposed cells, with clustering of individual treatment groups and cell lines being evident. Initial data gained following Crocidolite fibre exposure indicates significantly decreased levels of tetradecane, tridecane and dodecane in NCI-H28 cells. Dodecane has previously been implicated as a potential breath based biomarker for lung cancer when it is increased [3], which suggests that asbestos exposure may influence mesothelial cell VOC profile in a way which differentiates it from lung cancer.

Conclusions: For the first time, this study has charcterised the VOC profile of MPM cell lines following asbestos fibre exposure. Data may be clinically relevant in the pursuit of developing a breath test for MPM, these specific VOCs could act as early biomarkers for MPM, which would enable the early diagnosis of MPM patients.

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P30

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Towards targeted exhaled breath analysis for young children in CF care to detect bacteria in the lungs

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Background: Children with Cystic Fibrosis (CF) are prone to bacterial infections, which can be associated with lung function decline. Culture dependent diagnosis of such infections is a challenge in paediatric patients due to limited sample availability and quality. As an alternative, exhaled breath analysis measuring volatile organic compounds (VOCs) might be a promising non-invasive tool to identify bacteria. Several explorative studies have suggested that VOCs can differentiate between bacterial species. The next step forward for exhaled biomarkers would be to move from discovery studies towards hypothesis driven targeted analysis, an essential step for clinical application. We aimed to test the feasibility of targeted exhaled breath analysis in young children with CF for the detection of Staphylococcus aureus (SA) using previously identified VOCs.
Methods: Children with CF aged 1-6 years were included. Breath samples were taken, stored in thermal desorption tubes and analysed by gas chromatography – mass spectrometry (GC-MS). Microbiological cultures were collected on the same day. After a literature review to identify SA related VOCs, a targeted peak detection via AMDIS (v.2.68) was performed on the GC-MS dataset. The resulting peak intensities were used for a multivariate modelling by Sparse Partial Least Squares Discriminant Analysis (sPLS-DA), after which the predicted outcomes (SA positive/negative) were compared with the actual microbiological outcomes.
Results: Exhaled breath collection was successful in 20/24 patients (median age = 4 yrs. (IQR: 2-6 yrs.)). We identified 5 VOCs associated with SA from the literature review (≥2 consistent notations). These VOCs were used to predict culture positivity for SA resulting in an accuracy of 70%, specificity of 89% and a sensitivity of 55%. The positive and negative predictive value of the test was 86% and 62%, respectively.
Conclusion: We showed that exhaled breath collection for targeted analysis is feasible in young children, and that VOC's associated with SA were detectable. One of the limitations of this study is the small sample size and that most microbiology cultures were taken by a nasopharyngeal swab, which may explain the low sensitivity of the model. To conclude, more research should be done to evaluate this methodology in larger cohorts.

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P31

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Utilising computational methods to determine an idealised lung clearance index

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Background: There is increasing clinical interest in the lung clearance index (LCI), a measurement derived from multiple breath washout (MBW) protocols. LCI is primarily seen as a measure of ventilation inhomogeneity which can act as a sensitive measure of disease progression; LCI has particular utility in the disease management of cystic fibrosis (CF).[1,2] However, there are a number of practical and conceptual limitations of the technique including significant variation due to measurement technique and the use of a single value to assess lung physiology.[3] This one-dimensional approach is attractive but may lack specificity when assessing patients with different underlying pathology.[4]

Methods: A N2 MBW is performed by the participant, a computational model of the lung is fit to these data using a regression-based optimisation routine.[5] The resulting model lung reflects the physiology of the individual, allowing the simulation of a MBW protocol performed with a theoretical gas which is entirely insoluble in biological tissue. From this simulation, an idealised LCI (iLCI) can be determined. By using the model lung, this simulation can be carried out multiple times with different aspects of the lung physiology altered in a way that is not possible experimentally. This allows the breakdown of the iLCI into contributions from the sources of lung inhomogeneity included in the lung model.

This novel technique has been used in a cohort of 20 adult subjects including CF patients and healthy controls.

Results and Conclusions: All 20 participicants returned iLCI values which correlate well with LCIs measured using existing methods. Indeed, the iLCI shows better separation of disease and healthy groups. The key advantage of the iLCI is the breakdown of the single value into contributions from different sources of inhomogeneity within the lung, not just ventilation inhomogeneity which is typically used to explain LCI values. It can be seen in these results that two similar iLCI values can have significantly different breakdowns suggesting different underlying pathology.

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P32

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The volatilome of human pluripotent stem cells using selected ion flow tube-mass spectrometry

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Background: Human pluripotent stem cells (hPSCs) proliferate indefinitely and produce all cell types. This has tremendous applications in the biomedical field. Large amounts of hPSCs are needed for such purposes, which require prolonged in vitro culture. However, hPSCs frequently acquire abnormal changes in long-term culture, and routine characterisation is needed to ensure the maintenance of a healthy state. The presence of hPSCs in cell therapies also has safety concerns, as these cells can form tumours. Characterisation and identification of hPSCs can be done by measuring their metabolism. Volatile organic compounds (VOCs) are metabolites that offer information regarding cellular metabolic activities. A strategy to detect VOCs is selected ion flow tube-mass spectrometry (SIFT-MS). SIFT-MS analyses gaseous samples for several compounds in real-time. Here, we used SIFT-MS to study the VOC profile of various hPSCs at different culture timepoints.

Methods: hPSCs media was collected on days 1 and 3 post-seeding and transferred into glass bottles for SIFT-MS. The bottles were purged with sterile air and incubated for 16 hours at 37°C. The headspace was analysed using multiple ion monitoring (MIM) mode with H_3O^+ precursor ion for 50 seconds.

Results: MIM data showed that different hPSCs emit and consume specific VOCs at distinct levels, dependent on colony confluency. hPSCs also expressed pluripotent markers, further validating the results observed from SIFT-MS.

Conclusions: The distinct VOC profile for each hPSC line reflects the inherent variability associated with hPSCs, and this was observed without apparent changes in protein expression. Thus, SIFT-MS identifies subtle changes in hPSCs behaviour that pass unnoticed with other techniques. SIFT-MS also provides detailed information non-invasively, which is advantageous over other non-invasive characterisation methods. SIFT-MS is a valuable resource in the monitorisation of hPSCs for cell manufacturing and clinics.

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P33

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Correlation of breath metabolites and microbiome in IBS population

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Background: Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder that does not show any structural or biochemical gastrointestinal alterations. Despite that patients are classified based solely on self-reported physiological symptoms such as constipation, diarrhea, mixed, or undefined subtypes. Recent studies have shown that fecal microbiome composition and breath volatile metabolites are associated with IBS [1–4], and thus, can serve as a potentially more precise phenotype of IBS patients. Yet, the connection between breath metabolites and microbiome are yet to be established. Therefore, the aim of the current study is to investigate the possible correlation between breath volatile metabolites and microbiome in the IBS population and their potential in finding new ways of phenotyping it.

Methods: In this study fecal microbiome and exhaled breath data of 103 IBS patients were used. It included measurements of 16 breath volatile metabolites and 2085 metagenomic shotgun sequencing of fecal samples along with clinical and nutritional information. After preprocessing, canonical correlation analysis (CCA) was used to investigate the correlation between breath metabolites and microbiome and to find the most relevant microbiome spices contributing to this correlation. In the consequent step, unsupervised Random Forest was applied to a combined data of microbiome and 16 breath metabolites. The presence of potential groupings with respect to other clinical and nutritional information was investigated using principal coordinates analysis (PCoA).

Results: CCA demonstrated significant correlation between 16 breath metabolites and 33 microbiome species in the IBS population (R = 84, p-value = 0.05). The top three bacteria species contributing to this correlation are two Firmicutes phyla species: *R. bicirculans*, *R. intestinalis* and one of Bacteroidetes phylum, *B. vulgatus*. Interestingly, *R. bicirculans* has enzymatic capability to utilize hemicelluloses (beta-glucans and xyloglucans) [5]. *R. intestinalis* was found to play a role in IBD patients, while *B. vulgatus* was experimentally confirmed to protect against colitis [6]. The PCoA of joined breath and microbiome data did not show any obvious groupings with respect to nutritional and clinical data.

Conclusions: The following step would be linking the clinical and nutritional data to microbiome and breath metabolites datasets. As the relationship can be bilateral as clinical symptoms and nutritional habits can be both results and cause of microbiome composition.

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P34

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Development and validation of a proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) method for analysis of short-chain fatty acids (SCFAs) in exhaled breath

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Background: Short-chain fatty acids (SCFAs) are important metabolites produced by the gut microbiome because of the fermentation of non-digestible polysaccharides. The most abundant SCFAs are acetic acid, propionic acid, and butyric acid which make up 95% of this group of metabolites in the gut. Monitoring SCFAs could therefore potentially provide information on the quality (fiber content) of an individual's diet. Current analytical methodologies for SCFA analysis do not allow real-time monitoring which could be beneficial for e.g. dietary intervention studies. Here, we developed a proton transfer reaction – time-of-flight – mass spectrometry method for real-time analysis of the most abundant SCFAs in exhaled breath.

Methods: A PTR-TOF 8000 instrument was used throughout all experiments. Online measurements were performed via a commercial breath sampler (Loccioni®, Angeli di Rosora, Italy). Branching ratios were determined under dry and humid drift tube conditions. A full method validation (linearity, detection limit, quantification limit, repeatability) was performed for using chemical standards and exhaled breath samples.

Results: Each SCFA showed excellent linearity over a concentration range of 0-100 part-per-billion volume (ppbV). Detection limits were in the low ppbV range (<1 ppbV). Furthermore, online breath sampling repeatability was assesed using five consecutive breath of five healthy volunteers. For each SCFA, repeatability was within analytically acceptable limits (<15%).

Quantification results for PTR-ToF-MS were compared with the golden standard thermal desorption – gas chromatography – mass spectrometry (TD-GC-MS). Concentrations found with PTR-ToF-MS were compared to TD-GC-MS results. An excellent correlation was found between

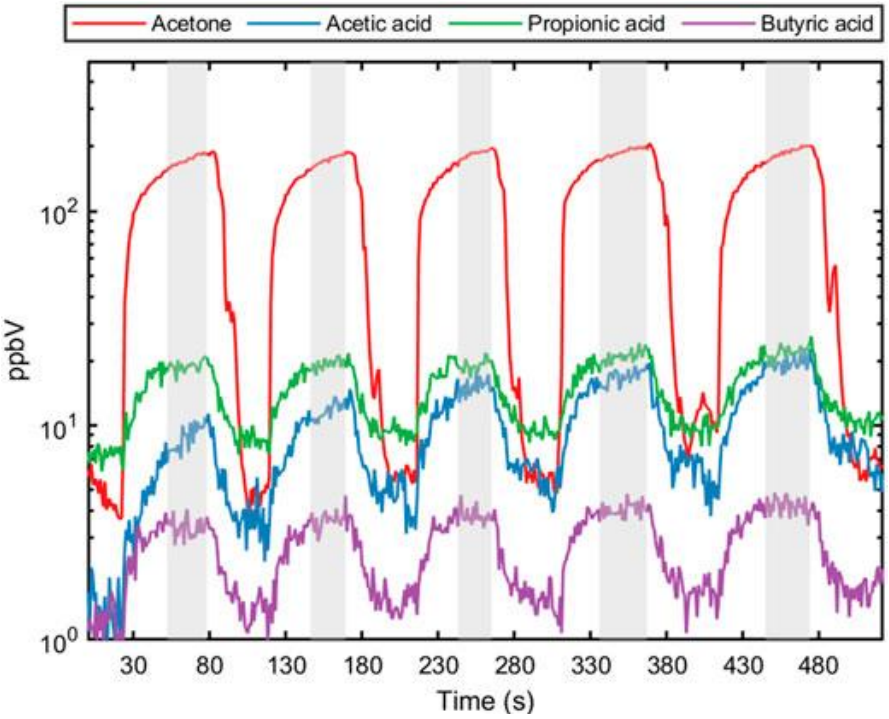

Fig. 5. Five consecutive exhalation profiles for acetone (breath marker) and each short-chain fatty acid (acetic acid, propionic acid and butyric acid) from a healthy individual. The shaded area represents the end-tidal part of the exhaled breath.

concentrations measured with PTR-ToF-MS and TD-GC-MS indicating that PTR-ToF-MS performs as good as the golden standard technique.
Conclusions: A method for SCFA analysis in exhaled breath was developed and validated. This opens new opportunities for non-invasive monitoring of SCFAs in exhaled breath during e.g. dietary intervention studies on children.

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P35

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Correlation of breath and blood cannabis levels using custom-made breath sample collection and analysis method

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Background: Cannabis legalization has occurred in many jurisdictions, with resultant need for a simple method to detect recency of use for the workplace and law enforcement. This pilot study shows the capability of the Cannabix Breath Analysis System to collect breath samples in the field and analyze them without complex sample preparation. Comparison between breath and gold standard blood Δ9-tetrahydrocannabinol (THC) levels was performed.
Methods: 6 consenting volunteers (2 women and 4 men, average age 23 ± SD 1.8 years) with history of cannabis use, smoked up to 1.4 grams (mean 0.9 ± SD 0.3) of THC ad libitum. Breath and blood samples were taken at baseline (prior to smoking) and at incremental time points out to 100 minutes after smoking. Measured breath samples were collected by a handheld Cannabix Breath Collection Unit (BCU) with a saliva trap to prevent oral fluid contamination. Collected samples were shipped on ice (40 hours) prior to analysis. Analysis was conducted using a Cannabix Breath Sampler coupled to Thermo TSQ Quantum Ultra mass spectrometer. THC fragments 123 m/z, 193 m/z, and 259 m/z were analyzed by measuring the area under the chromatogram curves (AUC) with Thermo Tune Master (Series 2.3 SP3) software. Blood samples were collected in red top tubes, centrifuged, with the time stamped

samples aliquoted and stored frozen prior to analysis using LC/MS/MS, by forensic laboratory standard operating procedures.

Results: Data demonstrates correlation between breath samples and blood plasma levels of THC (Fig. 1). Furthermore, the data supports the literature[1] by showing the same concentration versus time trends for THC metabolism assessed from breath and blood.

Conclusions: We successfully tested novel technology for detection of THC in human breath using a BCU and laboratory MS Breath Sampler. The technique has the potential to permit remote analysis of breath samples, significantly decrease laboratory analysis time, while maintaining sensitive, precise results. Furthermore, adding FAIMS (High-Field Asymmetric Waveform Ion Mobility Spectrometry) to isolate THC or other poorly volatile molecules, this method can be made portable for a real-time point-of-care testing. However, larger studies are needed to confirm the results.

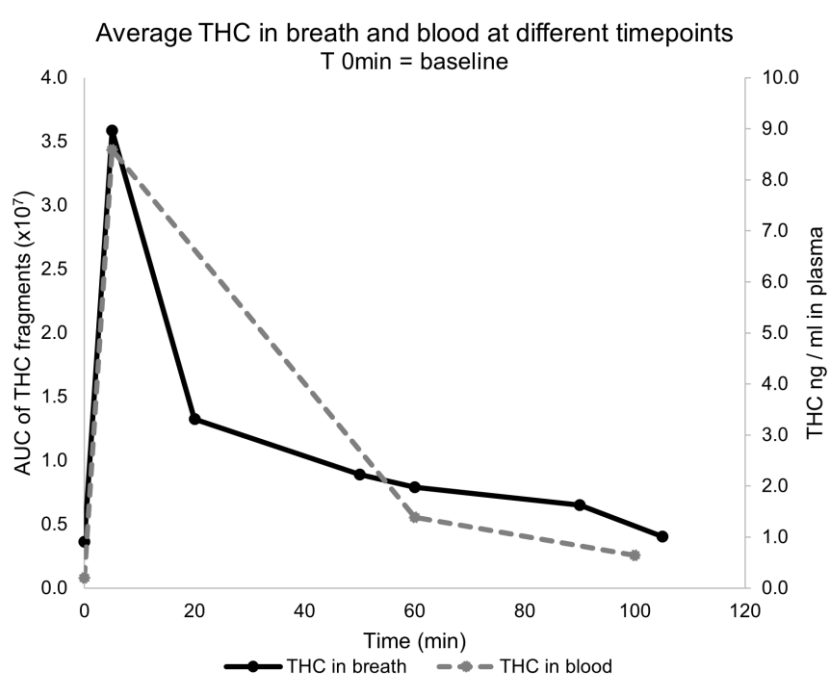

Fig. 1. Average Δ9-THC levels in breath and blood for all subjects.

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P36

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Monitoring dietary status and cognitive functioning in children through exhaled breath analysis

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Background: The main goal of this study was to examine the relation between exhaled breath biomarkers and diet quality for children with various dietary habits and cognitive abilities. The gut microbiome plays a crucial role in the host's immune system [1]. In particular, gut microbiome is vital in preventing microbial translocation across the epithelial/intestinal barrier [2]. There is evidence that unhealthy diets can elevate inflammatory tone by increasing gut permeability and introduce changes in gut microbiome. A number of studies have demonstrated that breath samples can be used to evaluate systemic inflammation in humans [3]. Consequently, analyzing the relationship between breath and nutritional status it could be beneficial, especially since it provides a non-invasive approach for detecting inflammatory conditions.

Methods: Duplicate breath samples were collected off-line from 32 children and analyzed with PTR-ToF-MS. For each child, anthropometric data and data on dietary intake (carbohydrates, fibers, etc.) were recorded. Cognitive functioning of the participating children was assessed through a Progressive Ratio Task (PRT) assessment (i.e. video game). Random Forest regression was used to identify volatile organic compounds (VOCs) and nutritional values indicative for the PRT results. Furthermore, correlation analysis (Spearman rank correlation) was used to investigate any potential link between dietary status and breath VOCs.
Results: In total, 6 VOCs (acetaldehyde, ethanol, acetic acid, dimethylsulfide, propionic acid and butyric acid) and two nutritional values (total fat intake and saturated fat intake) were found to be important for the Random Forest regression model (out-of-bag importance > 0.1). Ethanol, acetic acid, propionic acid and butyric acid were all positively and significantly correlated with the PRT results, whilst acetaldehyde, total fat intake and saturated fat intake showed significant and negative association with the PRT outcome.
Conclusions: Significant correlations were found between breath VOCs, nutritional values and spatial cognitive performance. These preliminary results show that non-invasive monitoring of dietary status/cognitive functioning in children through exhaled breath analysis could be a valuable tool that needs to be further explored and validated in large study cohorts.

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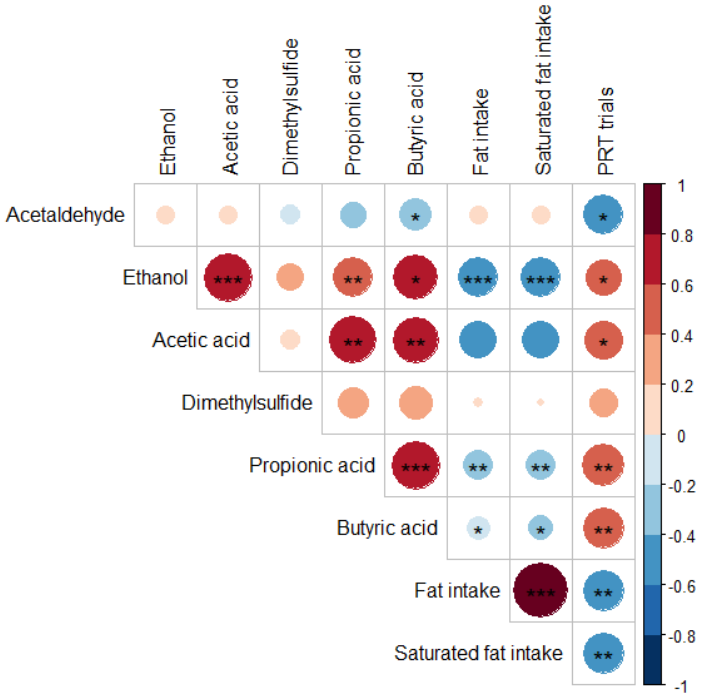

P37

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A feasibility study using the ETL CoronaCheck® to identify incident cases of SARS-CoV-2: Find SARS-CoV-2

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Background: The ETL CoronaCheck® is a hand-held, portable, point of care device which allows safe collection of exhaled breath condensate and provides an automated platform to detect SARS-CoV-2. We compared the ETL CoronaCheck® outcome to the current gold standard polymerase chain reaction (PCR) testing on nasopharyngeal swabs.
Methods: A cross-sectional study was performed comparing four groups of participants. Cohort A were test participants stratified by symptoms and PCR result. Cohort B was a prospective real-world evaluation of participants attending for PCR testing. Each cohort included 4 groups: Grp 1 symptomatic + PCR positive, Grp 2 symptomatic + PCR negative, Grp 3 asymptomatic + PCR positive, Grp 4 asymptomatic + PCR negative.
Results: 385 participants were recruited with 214 participants achieving a sample. Various device modifications during the trial improved the sample success rate. Cohort A included 60 participants (28%) in Grp 1, 31 (14.5%) in Grp 2, 22 (10.3%) in Grp 3 and 66 (30.8%) in Grp 4. Cohort B included 1 (0.5%) in Grp 1, 4 (1.9%) in Grp 2, 0 in Grp 3 and 30 (14%) in Grp 4. Combining the ETL CoronaCheck® outcomes from cohorts A and B gives an overall sensitivity of 99%, specificity 96%, positive predictor value 94%, negative predictor value 99% and accuracy 97%.
Across all participants with successful samples, there was excellent predictability between PCR and ETL CoronaCheck® with low variability, ROC: 0.98 (CI: 0.94–1.00). Cohen's K score confirmed excellent agreement between a positive vs negative ETL CoronaCheck® outcome against a positive vs negative PCR results (k=0.990, 99%, SE:0.10). 197 (92%) participants reported ETL CoronaCheck® easy to use, highlighting the usefulness in both symptomatic and asymptomatic participants.

Conclusions: The ETL CoronaCheck® is an easy to use, patient friendly and accurate point of care investigation which could be used in hospital and community settings for the screening and diagnosing SARS-CoV-2.

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P38

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Exhaled volatile organic compounds in suspected asthma - preliminary data from the RADicA study

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Background: Asthma is prevalent and the misdiagnosis rate substantial. Whilst exhaled volatile organic compounds (VOCs) demonstrate distinct profiles in patients with asthma compared to healthy volunteers, their performance at diagnosis, i.e. in symptomatic and treatment-naïve patients who have clinician-suspected asthma, remains unknown. We aim to investigate the role of VOCs in asthma diagnosis.

Methods: Symptomatic and untreated patients with GP-suspected asthma were recruited. Clinical history was taken and physical examination carried out. Asthma diagnostic tests, including fractional exhaled nitric oxide, spirometry with bronchodilator reversibility, peak expiratory flow monitoring and bronchial challenge were performed. Patients were skin prick tested and blood eosinophil levels measured. Inhaled corticosteroids (ICS) were initiated and tests repeated after 1-8 weeks. Exhaled VOCs were collected at baseline and following treatment. Duplicate breath samples were collected for reproducibility assessment, with matching tube and marks background samples. Asthma diagnostic outcomes were confirmed or refuted based on all available clinical evidence by at least two asthma specialists.

Results: We will present important aspects of study design relevant to other diagnostic VOC settings. As of April 2022, 257 patients (median [IQR] age: 21.8 [8.7-34.5] years, 42.4% male) were consented into the study, of which 222 had asthma diagnositic outcome confirmed (52% asthma, 28% not asthma, and 20% unclassified). Of 637 VOC-sampling procedures successfully performed, 378 were sampled pre-ICS and 259 of these were sampled again post ICS. Fifty-seven (8%) VOC collections procedures were unsuccessful, only two due to the patient being unable to perform.

Conclusions: In this ongoing large diagnostic study in people with suspected asthma aged 5 and over, breath VOC sampling is feasible and well tolerated. The ongoing analysis will elucidate the role of VOCs in asthma diagnosis in adults and children.

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P39

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Generating pooled quality control samples for VOC analysis by TD-GC-MS

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Quality control samples (QCs) are strongly recommended by the metabolomics community as representative samples for reproducibility measurements and to assess analytical drift [1]. Current methods for generating representative QC samples of volatile organic compounds (VOCs) include mixing chemical standards or collecting multiple samples in a gas collection bag or container. However, these methods are not ideal for longitudinal and multi-centre studies where samples are pre-concentrated onto thermal desorption (TD) tubes [2].
In this study, we make use of a tube stacking feature incorporated in a TD autosampler (TD-100, Markes International, UK) to generate pooled QC samples at the time of analysis. Samples were sequentially split after desorption into a secondary "pool" tube. All samples and QCs were analysed by TD-GC-MS. We applied this method to three separate studies of exhaled breath, bacterial headspace, and external standard mixture samples. Principal component analysis was performed to assess how well the QCs represented intra-study sample groups. Our preliminary results show that each intra-study sample group is qualitatively represented in pooled QCs. Sorbent artefacts and split ratios must be considered for future use. We demonstrated proof-of-principle for generating pooled QC samples for longitudinal and multi-centre studies, particularly useful in untargeted volatilomic studies.

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P40

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Direct thin-film microextraction of volatile metabolites from an air-liquid interface culture infected with *Staphylococcus aureus*

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Early detection of lung infection is critical to clinical diagnosis, treatment, and monitoring [1]. Measuring volatile organic compounds (VOCs) in exhaled breath has shown promise as a rapid and accurate method of evaluating disease metabolism and phenotype. However, further investigations of the role and function of VOCs in bacterial-host-stress response is required and this can only be realised through representative in-vitro models.

In this study we sample VOCs from the headspace of Transwell air-liquid interface (ALI) cultures using a novel thin film microextraction method in order to profile the volatilome of S. aureus during in colonisation on human alveolar epithelial cells. VOCs were analysed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS).

With an optimised ALI culture infection method, statistically significant VOCs were extracted from supervised principal component-discriminant function analysis, some of which were also found in axenic S. aureus cultures.

Our findings show that VOCs may be unique to infection and presence of S. aureus, and therefore suggest the capability of VOC analysis as a highly sensitive and specific test for S. aureus lung infection.

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P41

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Volatile metabolite profiling of morphological changes in *Candida albicans*

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Candida albicans is a versatile fungus of multiple organs including lungs. It is known for its ability to change between yeast and hyphal forms based on environmental stimuli. The sesquiterpenoid farnesol has shown to regulate this dimorphic switch [1] . However, additional volatile organic compounds (VOCs) may also be involved in filamentation. In this study we will apply an untargeted approach to profile the volatilome of *C. albicans* cultures under conditions which promote filamentous growth. A method was developed to passively sample headspace above C. albicans cultures using PDMS probes (Hisorb, Markes International, UK). VOCs were analysed by Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS). Data were pre-processed and VOCs of interest extracted using from principal component analysis loadings. 370 VOC features were extracted. Farnesol was identified in the headspace of *C. albicans*, as well as other characteristic fungal compounds such as 3-methylbutanal, and phenylethyl alcohol. Our results show that VOCs were able to discriminate between yeast and hyphae morphologies. Although influences such as pH, media, and interactions with the host require further investigation, results from this study may be useful to develop a diagnostic test for the invasive hyphae form of *C. albicans*.

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P42

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Recommended methods for safe breath analysis under highly infectious respiratory conditions

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Background: Being the proximal matrix, exhaled breath offers immediate metabolic outlook of respiratory infections. However, high viral load in exhaled breath imposes higher transmission risk that shouts for further optimizations of sampling and analytical methods without compromising the volatile metabolic marker exhalations [1,2].
Methods: Here, we have optimized our methods for online and offline mass-spectrometry based breathomics in patients with SARS-CoV-2 and/or similar respiratory infections. To reduce transmission risk, certain mainstream and side-stream viral filters are tested for online and offline applications. Confounders from filters, effects of operational conditions were assessed.
Results: We observed both physiological and analytical effects of the unavoidable COVID-19 safety measures on exhaled breath biomarkers resulting in immediate changes in VOC contractions. Such effects were substance specific and depended on the origin and physiochemical properties of VOCs. Immediate physiological effects were observed in case of main-stream viral filters due to implied upper-airway resistance against breathing. In case of side-stream syringe-filters, only analytical effects were observed and those effects were systematic. Effects partially depended on the sampling flow and respiratory rate. For syringe sampling factors such as storage time, -conditions and -temperature played important role.
Conclusions: Our methods are able to provide repeatable conditions for point of care and lab-based breath sampling and analysis with low risk of disease transmission under COVID-19 and similar infectious pathogens. Besides breath VOC profiling in spontaneously breathing subjects (healthy, asymptomatic positive and mildly-symptomatic patients) under the screening scenario of a COVID-19 test centre, the above protocol is applicable for moderately-symptomatic (with SARS onset) and/or severely ill mechanically-ventilated patients at the COVID-19 intensive care unit (ICU).

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Index

Session numbers	
1	From Crete to Pisa – two decades of breath research
2	Breath biomarkers of Sars Cov-2 infection
3	Spectroscopic methods for breath analysis
4	Sensors and sensor systems
6	Breath biomarkers of microbial infections
7	Breath tests targeting unmet clinical needs
8	Data analysis and interpretation
9	Analytical methods for breath biomarker detection
10	Breath analysis and cancer research
11	Five shades of breath analysis
12	Volatile signatures of asthma in breath
13	Young scientist session I
14	Young scientist session II
15	Skin volatilome and other stories

Abbreviations:

PLEN – Plenary Lecture

IT – Invited talk

O – Oral presentation

P – Poster

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