INTERNATIONAL ASSOCIATION OF BREATH RESEARCH
BREATH SUMMIT 2019
8-11TH SEPTEMBER 2019
CONFERENCE PROGRAMME AND ABSTRACTS
JBR has recently launched two special issues now open for submissions. Most content will be invited by the Guest Editors, but if you have a paper you would like to be considered for one of these ongoing issues please email the journal mailbox at jbr@ioppublishing.org.

**Breath Analysis in Perioperative and Critical Care Medicine**
**Guest Editors**
- Nandor Marcin, Harefield Hospital, UK
- Simona Cristescu, Radboud University, Nijmegen, The Netherlands
- Stephen Fowler, The University of Manchester, UK
- Marcus J Schultz, University of Amsterdam, The Netherlands

**Optical Spectroscopy for Breath Analysis**
**Guest Editors**
- Simona Cristescu, Radboud University, Nijmegen, The Netherlands
- Torsten Frosch, Leibniz Institute of Photonic Technology

Visit [iopscience.org/jbr](http://iopscience.org/jbr) for more information
Table of Contents

Sunday 08 September 2019 .......................................................... 9
THE TURING EXHIBITION SPACE .......................................................... 9

Monday Morning 09 September 2019 ............................................... 11
THE TURING EXHIBITION SPACE .......................................................... 11

Monday Morning 09 September 2019 ............................................... 13
THE STEPHENSON LECTURE ROOM .................................................. 13

Monday Afternoon 09 September 2019 .............................................. 14
THE TURING EXHIBITION SPACE .......................................................... 14

Monday Afternoon 09 September 2019 .............................................. 15
THE STEPHENSON LECTURE ROOM .................................................. 15

Tuesday Morning 10 SEPTEMBER 2019 ........................................... 17
THE TURING EXHIBITION SPACE .......................................................... 17

Tuesday Morning 10 SEPTEMBER 2019 ........................................... 19
THE STEPHENSON LECTURE ROOM .................................................. 19

Tuesday Afternoon 10 SEPTEMBER 2019 ........................................... 20
THE TURING EXHIBITION SPACE .......................................................... 20

Tuesday Afternoon 10 SEPTEMBER 2019 ........................................... 21
THE STEPHENSON LECTURE ROOM .................................................. 21

Wednesday 11 SEPTEMBER 2019 ...................................................... 23
THE TURING EXHIBITION SPACE .......................................................... 23

Sunday 08 September 2019 .......................................................... 25
THE TURING EXHIBITION SPACE .......................................................... 25

1. PLENARY: VOC Analysis - A Trailblazer In Translational Research? ..... 26

2. PLENARY: Analyzing Volatile Organic Compounds For The Detection Of Colorectal Cancer .......................................................... 27

Monday 09 September 2019 .......................................................... 29
THE TURING EXHIBITION SPACE .......................................................... 29

3. PLENARY: VOCS Related To Systemic Inflammatory Response Syndrome In A Caprine Animal Model .......................................................... 31

4. HEADLINE: Characterization of VOCs Emitted from Pathogenic Bacteria and Algal Cultures Using SPME-GC-MS: Towards Non-Invasive Diagnostics for Biosecurity and Bioenergy .......................................................... 32

5. Identifying Volatile Biomarkers For A Valley Fever Breath Test ........ 34

6. Identification Of The Most Prevalent Pathogens In Abdominal Sepsis Based On Headspace Analysis Of Volatile Organic Compounds ................ 35

7. HEADLINE: VOC analysis for metabolic monitoring in vitro............... 36

8. Detection of Mycobacterium avium ssp. paratuberculosis from native samples using VOC analysis and machine learning tools .................... 37

9. VOC profiles mirror viral-bacterial (co)infections in human cells ....... 38
10. PLENARY  Beyond PTR - Additional ionization modes in PTR-TOFMS and their use in breath gas analysis..........................39
11. "Breath Intelligence" - A Portable Handheld System For The Monitoring Of Breath Compounds Related To Metabolism And Oral Hygiene............40
12. Further Evaluation of a Standardized Breath Sampling Device for Off-line Exhaled Breath Analysis................................................41
13. HEADLINE: Effect of wood smoke exposure on exhaled breath CO and pulmonary gas exchange parameters..............................42
14. Efficacy of 13C-breath test as a beacon to locate entrapped casualties in search and rescue missions ..............................................43
15. Identification of a large set of volatile organic compounds characteristic for cystic fibrosis in children ........................................44
16. Perioperative anesthesia analysis of volatile organic compounds (VOC) in plasma and urine for lung adenocarcinoma and pulmonary granuloma by HS-GC-IMS..................................................................................45

THE STEPHENSON LECTURE ROOM ........................................................................47

17. HEADLINE: Direct Detection Of Sub-ppbv Level Of Breath N-Alkanes By Photoelectron Induced O2+ Cation Chemical Ionization Mass Spectrometry..........................................................48
18. Using Labelled Internal Standards To Improve The Analytical Performance Of Breath Analysis By Needle Trap Micro-Extraction Gas Chromatography-Mass Spectrometry (NTME-GC-MS)..................................................49
19. Development Of An IMS-Based Method For Passenger Control At Airports: A Proof-Of-Concept Study ........................................50
20. HEADLINE: Automated Thermal Desorption (TD)-SIFT-MS: A New Paradigm for Breath Analysis..................................................51
21. Taking Soft Chemical Ionisation Mass Spectrometry techniques on the “Walk of the World” a breath-taking adventure ..............................................52
22. Selective and Sensitive Measurement of Trace Exhaled HCN by Acetone-Assisted Negative Photoionization Time-of-flight Mass Spectrometry...53
23. ETNO As A Biomarker For Investigating The Effect Of Different OLV Strategies On Lung Injury And Inflammation Response ......................54
24. Stability of FENO50 in a COPD cohort in Sweden over 2-year follow-up55
25. HEADLINE: A proposed data standard for breath sample data and metadata.............................................................................56
26. Combining field-asymmetric ion mobility spectrometry (FAIMS), Infrared (IR) and luminescence sensing (LS) for artificial breath analysis ....57
27. Collection Of Breath Samples For Offline ‘Breathomics’ Mass Spectrometry Analysis Using The ReCIVA® Device In Patients With Acute Breathlessness: A Feasibility Study......................................................58
28. Real-time breath analysis with SESI-HRMS, confounding factors & standardization strategies to initiate multi-center studies......................60

Tuesday 10 SEPTEMBER 2019 .................................................................................61

THE TURING EXHIBITION SPACE ............................................................................61

29. PLENARY Utilizing the US-EPA CompTox Chemicals Dashboard to deliver public access to a Human Volatilome subset of data..........................62
HEADLINE: Standardization procedure for exhaled breath analysis using secondary electrospray ionization mass spectrometry ......................... 63
31. Saliva Screening For Rapid Organophosphate Poisoning Screening: A Case Study With Preliminary Observations And Findings .................. 64
32. Investigating chorioamnionitis in animal model by exhaled breath analysis .................................................................................. 66
33. HEADLINE: Baseline breath volatiles of healthy Non-Human Primates 68
34. Skin volatile profiling using gas chromatography-mass spectrometry as a means to track health status ........................................... 70
35. Untargeted Volatile Molecular Profiling of Human Breast Milk using HS-SPME-GC×GC-TOFMS for the Detection of Novel Metabolites .......... 71
36. PLENARY: Detecting Opioid Metabolites in Exhaled Breath Condensate (EBC) .............................................................................. 72
37. Human Exhaled Breath Condensate and Aerosol Collection in the Clinical Setting - Techniques, Concerns, and Considerations .......... 74
38. EBC analysis in a pre-clinical study: determining optimal analysis strategy .................................................................................... 75
39. HEADLINE: Study Design and Clinical Applications of Breath Analysis. 77
40. Developing Metrology Capabilities to Underpin Breath Analysis ........ 78
41. Diet and other factors affecting the variability of breath composition .. 79
THE STEPHENSON LECTURE ROOM ............................................................................................................................................. 80
42. HEADLINE: Real-time breath analysis during exhaustive exercise on a cycle ergometer ................................................................. 81
43. Circadian rhythm of exhaled biomarkers in health and asthma .......... 83
44. Determination of VOCs in breath samples and their potential to assess diagnosis of respiratory diseases .................................... 85
45. HEADLINE: EVOC Probes For The Assessment Of Metabolic Pathways: Using Breath Limonene To Assess The Impact Of Liver Disease .... 87
46. Stable isotope or unlabeled-probe breath tests vs endogenous VOC’s breath tests - a review ............................................................... 88
47. Fighting Anti-Microbial Resistance With Breath Analysis ................. 89
48. Modelling electronic nose sensor deflections by matching Gas Chromatography-Mass Spectrometry analysed exhaled breath samples ........................................................................................................ 90
49. Prospective Early Detection of Lung Cancer in COPD Patients by Electronic Nose Analysis of Exhaled Breath .................................... 91
50. HEADLINE: Role of Breath Based Volatile Organic Compounds in Detection of Gastroesophageal Disorders ............................... 93
52. Multimodal breath-based asthma phenotyping using GCxGC-HRTOFMS and SIFT-MS approaches .............................................. 95
Wednesday 11 SEPTEMBER 2019 ............................................................................................................................................. 96
THE TURING EXHIBITION SPACE ................................................................................................................................................. 96
53. Real-time therapeutic monitoring of valproic acid in exhaled breath .... 97
PTR-MS Solutions for Medical Breath Analysis

PTR-TOFMS Series
- real-time VOC breath analysis
- ultra-sensitive, soft ionization
- full high-resolution TOF spectrum in a split-second
- monitoring metabolic processes
- screening for disease markers

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SUNDAY 08 SEPTEMBER 2019

THE TURING EXHIBITION SPACE

Chaired by: C. L. Paul Thomas and Marieann Högman

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>15:00</td>
<td>IABR Chair Prof Marieann Högman</td>
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<tr>
<td>15:10</td>
<td>Welcome to Loughborough</td>
</tr>
<tr>
<td>15:20</td>
<td>Anton Amman Award and lecture</td>
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<td></td>
<td>To be advised</td>
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<tr>
<td>15:40</td>
<td></td>
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<tr>
<td>16:00</td>
<td>Refreshment and poster set up (The Babbage Room)</td>
</tr>
<tr>
<td>16:30</td>
<td>VOC analysis - a trailblazer in translational research</td>
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<tr>
<td>17:00</td>
<td>Wolfram Miekisch</td>
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<tr>
<td>17:00</td>
<td>Analyzing Volatile Organic Compounds for the detection of Colorectal Cancer</td>
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<td>Robert van Vorstenbosch</td>
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<tr>
<td>17:30</td>
<td>Your Conference Programme and Guidance</td>
</tr>
<tr>
<td>17:40</td>
<td>IABR Members meeting</td>
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<tr>
<td>17:45</td>
<td>Reception and Posters</td>
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The world-leading providers of SIFT-MS solutions

Multiple inlet options for breath analysis, including direct analysis and automated thermal desorption.
<table>
<thead>
<tr>
<th>Start</th>
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<th>Title</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>09:00</td>
<td>09:45</td>
<td><strong>VOCs related to systemic inflammatory response syndrome in a caprine animal model</strong></td>
<td>Elisa Kasbohm</td>
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<td>09:50</td>
<td>10:20</td>
<td><strong>Characterization of VOCs emitted from pathogenic bacteria and algal cultures using SPME-GC-MS: Towards non-invasive diagnostics for biosecurity and bioenergy</strong></td>
<td>Matthias Frank</td>
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<tr>
<td>10:20</td>
<td>10:40</td>
<td><strong>Identifying volatile biomarkers for a valley fever breath test</strong></td>
<td>Heather Bean</td>
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<tr>
<td>10:40</td>
<td>11:00</td>
<td><strong>Identification of the most prevalent pathogens in abdominal sepsis based on headspace analysis of volatile organic compounds</strong></td>
<td>Kim Hintzen</td>
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<tr>
<td>11:15</td>
<td>11:45</td>
<td><strong>VOC analysis for metabolic monitoring in vitro</strong></td>
<td>Ann-Christin Klemenz</td>
</tr>
<tr>
<td>11:45</td>
<td>12:05</td>
<td><strong>Detection of Mycobacterium avium ssp. paratuberculosis from native samples using VOC analysis and machine learning tools</strong></td>
<td>Philipp Vitense</td>
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<tr>
<td>12:05</td>
<td>12:25</td>
<td><strong>VOC profiles mirror viral-bacterial (co)infections in human cells</strong></td>
<td>Selina Traxler</td>
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CONFIRMED SPEAKERS

Steven Fowler  University of Manchester
George Hanna  Imperial College London
Jane Hill  Thayer School of Engineering, Dartmouth
Olaf Holz  Fraunhofer Institute for Toxicology & Experimental Medicine
Jessica Lasky-Su  Brigham and Women’s Hospital, Boston
Renaud Louis  University of Liege
Chris Mayhew  Institute of Breath Research, University of Innsbruck
Anil Modak  Owlstone Medical Scientific Advisory Board
Douglas Morrison  University of Glasgow
João Rufo  University of Porto
Jose Torrecilla  Complutense University of Madrid

owlstonemedical.com/breath-biopsy-conference

Owlstone Medical
<table>
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<tr>
<th>Time</th>
<th>Session</th>
<th>Chair by:</th>
<th>Title</th>
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<tr>
<td>09:50</td>
<td>Session 3</td>
<td>Michael Davis</td>
<td>Direct Detection of Sub-ppbv Level of breath n-alkanes by Photoelectron Induced O2+ Cation Chemical Ionization Mass Spectrometry Lei Hua</td>
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<td>10:20</td>
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<td>Using labelled internal standards to improve needle trap micro-extraction technique prior to gas chromatography/mass spectrometry Tommaso Lomonaco</td>
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<td>10:40</td>
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<td>Development Of An IMS-Based Method For Passenger Control At Airports: A Proof-Of-Concept Study Isabel Steppert</td>
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<tr>
<td>11:15</td>
<td>Session 4</td>
<td>Michael Wilde</td>
<td>Automated Thermal Desorption (TD)-SIFT-MS: A New Paradigm for Breath Analysis Nathan Hawkins</td>
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<tr>
<td>11:45</td>
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<td>Taking Soft Chemical Ionisation Mass Spectrometry techniques on the “Walk of the World” a breath taking adventure Ben Henderson</td>
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<tr>
<td>12:05</td>
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<td>Selective and Sensitive Measurement of Trace Exhaled HCN by Acetone-Assisted Negative Photoionization Time-of-flight Mass Spectrometry Haiyang Li</td>
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### THE TURING EXHIBITION SPACE

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<tr>
<th>Start</th>
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<th>Session 5</th>
<th>Chaired by: Pablo Sinues and Wolfram Miekisch</th>
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<tr>
<td>13:30</td>
<td>14:10</td>
<td>Beyond PTR - Additional ionization modes in PTR-TOFMS and their use in breath gas analysis</td>
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<td>Jens Herbig</td>
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<td>14:15</td>
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<td>Breath Intelligence - A Portable Handheld System For The Monitoring Of Breath Compounds Related To Metabolism And Oral Hygiene</td>
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<td>Ulrike Lehmann</td>
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<td>14:35</td>
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<td>Further Evaluation of a Standardized Breath Sampling Device for Off-line Exhaled Breath Analysis</td>
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<td>Sean Harshman</td>
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<th>Session 6</th>
<th>Chaired by: Anil Modak and Jonathan Beauchamp</th>
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**Session 7**  
**Chaired by: Agnieszka Smolinska**  
ETNO as a biomarker for investigating the effect of different OLV strategies on lung injury and inflammation response  
Yang Lyu  
14:15 | 14:35
--- | ---
Stability of FENO50 in a COPD cohort in Sweden over 2-year follow-up  
Marieann Högman  
14:35 | 14:55
--- | ---
A proposed data standard for breath sample data and metadata  
Bo Zhao  
15:30 | 15:55

**Session 8**  
**Chaired by: Chris Mayhew**  
Combining field-asymmetric ion mobility spectrometry (FAIMS), Infrared (IR) and luminescence sensing (LS) for artificial breath analysis  
Tamina Hagemann  
15:55 | 16:15
--- | ---
Collection of breath samples for offline ‘breathomics’ mass spectrometry analysis using the ReCIVA® device in patients with acute breathlessness: a feasibility study  
Karl Holden  
16:15 | 16:35
--- | ---
Real-time breath analysis with SESI-HRMS, confounding factors & standardization strategies to initiate multi-center studies  
Guillermo (William) Vidal de Miguel  
16:35 | 16:55
SUPER SESI
Relevant metabolites in real time
Simplify sample handling
Harnesses the power of HRMS

EXHALION
Guide and standardize
the exhalation maneuver

Breath Analysis Technology
<table>
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<tbody>
<tr>
<td><strong>Session 9</strong></td>
<td><strong>Chaired by: Simona Cristescu and Aoife Morrin</strong></td>
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<tr>
<td>09:00</td>
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<td>Utilizing the US-EPA CompTox Chemicals Dashboard to deliver public access to a Human Volatilome subset of data</td>
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<td>Joachim Pleil</td>
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<td>09:50</td>
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<tr>
<td>Standardization procedure for exhaled breath analysis using secondary electrospray ionization mass spectrometry</td>
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<td>Bettina Streckenbach</td>
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<td>10:20</td>
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<tr>
<td>Saliva screening for rapid organophosphate poisoning assessment: A case study with preliminary observations and findings</td>
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<td>Andria Hadjithekli</td>
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<td>10:40</td>
<td>11:00</td>
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<tr>
<td>Investigating chorioamnionitis in animal model by exhaled breath analysis</td>
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<tr>
<td>Agnieszka Smolinska</td>
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<tr>
<td><strong>Session 10</strong></td>
<td><strong>Chaired by: Haiyang Li and Chris Mayhew</strong></td>
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<tr>
<td>11:15</td>
<td>11:45</td>
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<tr>
<td>Baseline breath volatiles of healthy Non-Human Primates</td>
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<td>Jannatu Azmir</td>
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<td>11:45</td>
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<tr>
<td>Skin volatile profiling using gas chromatography-mass spectrometry as a means to track health status</td>
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<tr>
<td>Aoife Morrin</td>
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<tr>
<td>Untargeted Volatile Molecular Profiling of Human Breast Milk using HS-SPME-GC×GC-TOFMS for the Detection of Novel Metabolites</td>
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<td>Lili Kang</td>
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GCxGC and TOF MS with Tandem Ionisation®

Providing added analytical insight for improved biomarker discovery

**BenchTOF™** mass spectrometers offer an unbeatable combination of sensitivity, spectral quality, selectivity, speed and stability, which together deliver 'high-definition' mass spectrometry – a powerful approach to any GC-MS or GCxGC-MS application.

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### Session 11

**Chaired by: Tobias Bruderer**

- **09:50 - 10:20**
  - Real-time breath analysis during exhaustive exercise on a cycle ergometer
  - Giovanni Pugliese

- **10:20 - 10:40**
  - Circadian rhythm of exhaled biomarkers in health and asthma
  - Max Wilkinson

- **10:40 - 11:00**
  - Determination of VOCs in breath samples and their potential to assess diagnosis of respiratory diseases
  - Ileana Andreea Ratiu

### Session 12

**Chaired by: Ann-Christin Klemenz**

- **11:15 - 11:45**
  - EVOC Probes For The Assessment Of Metabolic Pathways: Using Breath Limonene To Assess The Impact Of Liver Disease
  - Rob Smith

- **11:45 - 12:05**
  - Stable isotope or unlabelled-probe breath tests vs endogenous VOC’s breath tests - a review
  - Anil Modak

- **12:05 - 12:25**
  - Fighting Anti-Microbial Resistance With Breath Analysis
  - Emma Brodrick
<table>
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<th>Finish</th>
<th>Session 13</th>
<th>Chaired by: Heather Bean and Norman Ratcliffe</th>
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<tbody>
<tr>
<td>13:30</td>
<td>14:10</td>
<td>Detecting Opioid Metabolites in Exhaled Breath Condensate (EBC)</td>
<td>Cristina Davis</td>
</tr>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Human Exhaled Breath Condensate and Aerosol Collection in the Clinical Setting - Techniques, Concerns, and Considerations</td>
<td>Michael Davis</td>
</tr>
<tr>
<td>14:35</td>
<td>14:55</td>
<td>EBC analysis in a pre-clinical study: determining optimal analysis strategy</td>
<td>Agne Krilaviciute</td>
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<th>Session 14</th>
<th>Chaired by: Agnieszka Smolinska</th>
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<tr>
<td>15:30</td>
<td>15:55</td>
<td>Study Design and Clinical Applications of Breath Analysis</td>
<td>Stephen Fowler</td>
</tr>
<tr>
<td>15:55</td>
<td>16:15</td>
<td>Developing Metrology Capabilities to Underpin Breath Analysis</td>
<td>Sergi Moreno</td>
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<tr>
<td>16:15</td>
<td>16:35</td>
<td>Diet and other factors affecting the variability of breath composition</td>
<td>Fabio Di Francesco</td>
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### Session 15

**Chaired by: Kerstin Wex**

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<tbody>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Modelling electronic nose sensor deflections by matching Gas Chromatography-Mass Spectrometry analysed exhaled breath samples</td>
<td>Paul Brinkman</td>
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<tr>
<td>14:35</td>
<td>14:55</td>
<td>Prospective Early Detection of Lung Cancer in COPD Patients by Electronic Nose Analysis of Exhaled Breath</td>
<td>Rianne de Vries</td>
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### Session 16

**Chaired by: Marcis Leja**

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<tbody>
<tr>
<td>15:30</td>
<td>15:55</td>
<td>Role of Breath Based Volatile Organic Compounds in Detection of Gastroesophageal Disorders</td>
<td>Ravi Vissapragada</td>
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<tr>
<td>16:15</td>
<td>16:35</td>
<td>Multimodal breath-based asthma phenotyping using GCxGC-HRTOFMS and SIFT-MS approaches</td>
<td>Pierre-Hugues Stefanuto</td>
</tr>
</tbody>
</table>
Various volatile compounds can be present in human breath depending on nutrition, metabolic state including diseases and medication, microbial infections and personal oral hygiene. Once authoritatively validated their presence and concentration represent valuable information sources for early diagnosis or therapy control. Several applications have been examined with scientific same as customer feasibility studies proving the power of this innovative non-invasive approach:

- monitoring endogeneous VOCs (e.g. aldehydes, ketones, alcohols, isoprene)
- research on potential marker compounds for diseases / infections measurement of drugs and their metabolites
- drug efficiency in pharmacokinetic studies
<table>
<thead>
<tr>
<th>Start</th>
<th>Finish</th>
<th>Session 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>09:20</td>
<td>Chaired by: C. L. Paul Thomas and Marieann Högman</td>
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<tr>
<td>09:20</td>
<td>09:40</td>
<td>Real-time therapeutic monitoring of valproic acid in exhaled breath</td>
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<td></td>
<td></td>
<td>Kapil Dev Singh</td>
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<td>09:40</td>
<td>10:00</td>
<td>The breath biomarkers of tuberculosis using model macaque monkey</td>
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<td></td>
<td>Mohammad Sharif Khan</td>
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<tr>
<td>10:00</td>
<td>11:45</td>
<td>A Clinical Breathomic Workflow For Metabolic Phenotyping</td>
</tr>
<tr>
<td>11:45</td>
<td>12:15</td>
<td>Dahlia Salman</td>
</tr>
<tr>
<td>12:15</td>
<td>12:30</td>
<td>Focus groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reflection Panel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prizes and closing comments with announcement of new officer(s)</td>
</tr>
</tbody>
</table>
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Register at congress.chestnet.org
15:00 15:10  IABR Chair Prof Marieann Högman
15:10 15:20  Welcome to Loughborough
15:20 16:00  Anton Amman Award and lecture
          To be advised
16:00 16:30  Refreshment and poster set up (The Babbage Room)
16:30 17:00  VOC analysis - a trailblazer in translational research
          Wolfram Miekisch
17:00 17:30  Analyzing Volatile Organic Compounds for the detection of Colorectal Cancer
          Robert van Vorstenbosch
17:30 17:40  Your Conference Programme and Guidance
17:40 18:00  IABR Members meeting
18:00 19:30  Reception
1. PLENARY: VOC Analysis - A Trailblazer In Translational Research?

Wolfram Miekisch

16:30

Miekisch W.,(1) Trefz P(1). Sukul P(1), Schubert JK(1).

(1) ROMBAT, Department of Anesthesiology and Intensive Care Medicine, University Medical Centre Rostock, Germany

In the last decades, VOC research focused on potential clinical claims – e.g. the (early) recognition of diseases such as cancer with limited success. A lack of basic understanding of VOC kinetics (generation, transport, distribution, excretion...) still hampers successful transfer of VOC research into application. Recent studies – e.g. in the field of real time monitoring – have shown the enlarged potential of VOC biomarkers for bridging the gap between basic understanding of (patho-)physiological processes and clinical research.

Based on two examples – focused on physiology and infection - this talk intends to demonstrate, how VOC analysis may be used for translational research.

Direct breath resolved measurement of complete VOC-profiles with time resolutions in the sub-sec ranges enables new insights into VOC exhalation and dependency of VOC profiles on physiological effects such as changes in hemodynamics or ventilation. These effects are not only useful to sort out confounding effects on VOC profiles but can also be used to gather physiological and metabolic insights from healthy volunteers such as information on anaerobic threshold or lung function.

VOC profiles may mirror infection course in vitro and in vivo. To gain a broader understanding on bacterial activity as well as on host response, VOC analyses are useful to monitor bacterial grow under well-defined conditions in vitro and effects of bacterial infection in well-controlled animal models. To transfer results into practice, field studies under realistic conditions must be performed. Practical application of such VOC tests are further fostered by innovative approaches such as crowd monitoring.

Due to the unlimited availability, the dynamic occurrence, the immediate reaction to interventions and the non-destructive nature, VOC analysis represents a unique monitoring tool in basic studies in vitro and in vivo. In animal models, the in vivo translation of potential in vitro marker compounds as well as the host response can be observed with only minimal influence of the analytical technique itself. Easy transfer of interesting research findings into field or clinical studies in a non-invasive way represents a further advantage of VOC based approaches. Due to these unique features, VOC analysis has the potential to act as a trailblazer in translational research.
Colorectal cancer (CRC) is one of the most prevalent diseases within the EU. The survival rate of CRC patients is dependent on the stage at which the disease is diagnosed. To facilitate early diagnosis, national screening programs are organized. In the Netherlands this consists of a non-invasive iFOBT test and, if tested positive, a follow-up colonoscopy. Unfortunately, this test suffers from low sensitivity and a high false positive rate. Therefore, a novel non-invasive diagnostics tool is urgently needed.

The analysis of Volatile Organic Compounds (VOCs) in exhaled breath might be a potential alternative, for they have previously been shown to correlate to cancer and cancer pre-stages. Therefore, the aim of this study is to demonstrate the feasibility of exhaled breath analysis to diagnose CRC and to compare its accuracy with the iFOBT test.

In this study, exhaled breath samples were collected from patients tested positive for the iFOBT test (n=410) by inflating a 3L Tedlar bags and transferring the contents within 1h to stainless thermal desorption tubes where VOCs were trapped. Later, they were analyzed using GC-tof-MS. The data was pre-processed using wavelets and p-splines to diminish effects of noise and baseline. After aligning the chromatograms, they were normalized by probabilistic quotient normalization.

The statistical analysis consisted of several steps. First, data was visualized using PCA, robust-PCA and unsupervised Random Forest. In the consequent step, the data was randomly divided into training (70%) and test set (30%). In order to find the CRC-specific volatile metabolites in exhaled breath, feature extraction was implemented in combination with linear and non-linear classification models including PLS-DA, tree based techniques and SVM. The prediction accuracy of each classification technique was accessed by means of precision-recall curve. This study shows that a specific set of volatile metabolites in breath enables the differentiation among CRC, pre-stages of cancer and healthy patients. Thus, it may prove to be of crucial importance in patients’ diagnosis and treatment processes. Exhaled breath analysis can therefore be a potential new non-invasive diagnostic and monitoring tool.
THE TURING EXHIBITION SPACE
## VOCs related to systemic inflammatory response syndrome in a caprine animal model
Elisa Kasbohm

- **Start:** 09:00
- **Finish:** 09:45

## Characterization of VOCs Emitted from Pathogenic Bacteria and Algal Cultures Using SPME-GC-MS: Towards Non-Invasive Diagnostics for Biosecurity and Bioenergy
Matthias Frank

- **Start:** 09:50
- **Finish:** 10:20

## Identifying Volatile Biomarkers For A Valley Fever Breath Test
Heather Bean

- **Start:** 10:20
- **Finish:** 10:40

## Identification Of The Most Prevalent Pathogens In Abdominal Sepsis Based On Headspace Analysis Of Volatile Organic Compounds
Kim Hintzen

- **Start:** 10:40
- **Finish:** 11:00

## VOC analysis for metabolic monitoring in vitro
Ann-Christin Klemenz

- **Start:** 11:15
- **Finish:** 11:45

## Detection of Mycobacterium avium ssp. paratuberculosis from native samples using VOC analysis and machine learning tools
Philipp Vitense

- **Start:** 11:45
- **Finish:** 12:05

## VOC profiles mirror viral-bacterial (co)infections in human cells
Selina Traxler

- **Start:** 12:05
- **Finish:** 12:25

## Beyond PTR - Additional ionization modes in PTR-TOFMS and their use in breath gas analysis
Jens Herbig

- **Start:** 13:30
- **Finish:** 14:10

## Breath Intelligence - A Portable Handheld System For The Monitoring Of Breath Compounds Related To Metabolism And Oral Hygiene
Ulrike Lehmann

- **Start:** 14:15
- **Finish:** 14:35

## Further Evaluation of a Standardized Breath Sampling Device for Off-line Exhaled Breath Analysis
Sean Harshman

- **Start:** 14:35
- **Finish:** 15:15

## Efficacy of 13C-breath test as a beacon to locate entrapped casualties in search and rescue missions
Makoto Sawano

- **Start:** 15:55
- **Finish:** 16:15

## Identification of a large set of volatile organic compounds characteristic for cystic fibrosis in children
Tobias Bruderer

- **Start:** 16:15
- **Finish:** 16:35

## Perioperative anaesthesia analysis of volatile organic compounds (VOC) in plasma and urine for lung adenocarcinoma and pulmonary granuloma by HS-GC-IMS
Enyou Li

- **Start:** 16:35
- **Finish:** 16:55

## IABR Members meeting

- **Start:** 17:00
- **Finish:** 17:20

## Election of Chair Elect
THE TURING EXHIBITION SPACE
Elisa Kasbohm

09:00

Kasbohm E (1), Redlberger S (2), Fischer S (2), Bergmann A (3)*, Miekisch W (3), Schubert JK (3), Köhler H (2), Reinhold P (2), Liebscher V (1)

(1) Institute of Mathematics and Computer Science, University of Greifswald, Greifswald, Germany;
(2) Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany;
(3) Department of Anaesthesia and Intensive Care, University Medicine Rostock, Rostock, Germany; * current address: Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland

Background

Under adverse conditions, a local inflammation may evolve into a systemic inflammation, a serious condition which is associated with organ dysfunction and high mortality. During the first weeks of a clinical experiment on mycobacterial infections in goats, one group of infected animals was unexpectedly affected by systemic inflammatory response syndrome (SIRS). Repeated VOC measurements of breath and fecal samples enabled us to study SIRS-related pathophysiological changes in detail.

Methods

The study comprised 18 goats experimentally inoculated with Mycobacterium avium subsp. hominissuis, 21 goats experimentally inoculated with Mycobacterium avium subsp. paratuberculosis and 10 goats as healthy control animals. Samples of exhaled breath were obtained in four-week intervals and fecal samples were obtained every two weeks. Breath samples were pre-concentrated by means of needle-trap microextraction (NTME) and analyzed by GC-MS. VOCs in headspace above fecal samples were pre-concentrated using NTME and solid phase microextraction (SPME), and subsequently analyzed by GC-MS. In addition, blood samples were collected every week and analyzed for blood electrolytes and serum proteins. For statistical analysis, we deployed random forests as a flexible, data-driven machine learning algorithm and included a robust feature selection method.

Results

23 VOCs in breath and 33 VOCs in headspace above fecal samples (33 for SPME, 31 for NTME) were found to be related to SIRS. 35 further variables (e.g. physiological parameters like weight gain, specific eicosanoids in blood) exhibited SIRS-related changes, which were partially highly correlated to VOCs in exhaled breath. In addition, we investigated potential prognostic markers for the fatality of SIRS.

Conclusions

By means of our flexible machine learning workflow, we were able to characterize SIRS-related VOC profiles for breath and feces in our animal model. These VOC profiles may yield further insight into underlying pathophysiological processes, in particular concerning SIRS-related blood markers as potential sources of VOC markers in exhaled breath. This would enable to describe specific VOC emissions using mechanistic modelling in future work.
Matthias Frank

09:50

Reese KL (1,2), Rasley A (1), Avila J (1), Jones AD (2,3), Fisher CL (4), Lane TW (4), Frank M (1)

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(2) Department of Chemistry, Michigan State University, 578 S Shaw Lane, East Lansing, MI 48824, USA
(3) Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, 48824
(4) Systems Biology Department, Sandia National Laboratories, Livermore, CA 94551 USA

Background

a) Pathogenic bacteria: A potential biosecurity-related application of breath analysis is to screen victims of biological attacks for exposure to a biological agent. This requires differentiation between the chemical markers from "normal, healthy" exhaled breath and potential breath markers produced by proliferating biological agents in the lung of exposed individuals. In this work we analyzed trace volatile organic compounds (VOCs) from Francisella tularensis novicida and Bacillus anthracis Sterne, both risk group 2 surrogates for potential biowarfare agents, as well as VOCs from the fully virulent B. anthracis Ames and F. tularensis SchuS4 grown under BSL-3 laboratory conditions.

b) Algae: Using a similar experimental approach as for bacteria, we also analyzed trace VOCs from algal cultures that are under study for bioenergy production. The goal was to investigate whether some VOCs produced by algae could be used as markers for the health status of cultures and/or early indicators that precede culture crashes due to pathogen or grazer infestation.

Methods

a) Pathogenic bacteria: F. t. novicida (strain U112) was cultured in modified Mueller-Hinton media, B. a. Sterne and B. a. Ames in Brain-Heart Infusion media. Volatile organic compounds (VOCs) emitted from several replicates of those cultures were sampled using solid-phase microextraction (SPME) at multiple time points and analyzed using gas chromatography-mass spectrometry (GC-MS). Individual compound identification was accomplished through comparison of chromatographically deconvoluted experimental spectra to library spectra (NIST14) and retention index matching. Control experiments included SPME VOC sampling of blank culture flasks and flasks with media only.

b) Algae and rotifers: Microchlororopsis salina cultures were grown in ESAW media in 20-liter polycarbonate carboys at room temperature. Algal culture density was monitored daily by chlorophyll fluorescence. 48 hours after inoculation, marine rotifers Brachionus plicatilis (grazers on algae) were added. At various time points, VOCs were sampled in duplicate from the headspaces of each culture and media control vessels using field-portable SPME fibers and analyzed by GC-MS. Compound identification was accomplished following a procedure similar to the work on bacteria.

Results
THE TURING EXHIBITION SPACE

a) Pathogenic bacteria: For both species of bacteria, F.t. and B.a., a number of characteristic VOCs were detected and attributed to the bacteria (not present in the controls). Bacterial VOC profiles were found to evolve over time as a function of bacterial growth state. Differentiation of the two species was accomplished through comparisons of the species-specific VOC fingerprints, reproducibly derived from multiple biological replicates. VOC fingerprints for B.a. Sterne and B.a. Ames showed some similarities, but also notable differences. Biological functions were attributed to validated biomarkers, e.g. odd-carbon numbered aliphatic methyl-ketones identified in F. t. novicida were attributed to metabolites formed during β-oxidation of fatty acids.

b) Algae: A number of characteristic VOCs were detected and attributed to the algae (not present in the controls). The addition of B. plicatilis to healthy cultures of M. salina generated, in addition, trans-β-ionone and β-cyclocitrail, which were attributed to carotenoid degradation. Rotifer-inoculated cultures displayed lower live algal counts relative to the uninoculated controls. The abundances of the carotenoid-derived VOCs increased with increasing rotifer consumption of algae.

Conclusions

a) Several VOC biomarkers from two species of bacteria closely related to potential biological agents and, more importantly, fully virulent species were identified. Our results indicate that breath analysis may have the potential to rapidly and non-invasively identify exposed individuals in a triage setting after a biological attack.

b) Several VOC biomarkers from healthy algal cultures as well as additional markers from infected cultures were identified. Our results indicate that specific VOCs released by infected algae cultures may be early indicators for impending pond crashes, providing a useful tool to monitor algal biomass production and help prevent pond crashes.
Identifying Volatile Biomarkers For A Valley Fever Breath Test

Heather Bean

10:20

Emily Higgins Keppler (1,2), Heather Mead (3,4), Bridget M. Barker (3,4), Heather D. Bean (1,2)

(1) School of Life Sciences, Arizona State University, Tempe, AZ;
(2) Center for Fundamental and Applied Microbiomics, The Biodesign Institute, Tempe, AZ;
(3) Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ;
(4) The Pathogen and Microbiome Institute, Flagstaff, AZ

Background

Valley fever (coccidioidomycosis) is an endemic fungal pneumonia of the North American and South American deserts. The current diagnostics for Valley fever are severely lacking due to poor sensitivity (via serology) and invasiveness (via biopsy), leading to delayed diagnosis, inappropriate treatment with antibiotics, lost productivity, and increased medical costs. There is a critical need for sensitive and non-invasive diagnostics for detecting and identifying Valley fever lung infections. Our long-term goal is to substantially shorten the time-to-diagnosis for Valley fever through the development of sensitive and specific breath-based diagnostics for coccidioidomycosis lung infections. In the near-term, we are working toward identifying volatile biomarkers of Coccidioides posadasii and C. immitis infections via metabolomics analyses of in vitro cultures, murine model lung infections, and lung specimens from humans with Valley fever. Herein we present recent data on the volatile profiles of C. posadasii and C. immitis grown in vitro as spherules and as mycelia.

Methods

Six strains of C. posadasii (three AZ and three TX/MEX/SA population strains) and six strains of C. immitis (three SJV and three SDMX strains) were cultured in triplicate for 96 h in RPMI 1640 with 10% FBS media at 39°C in 10% CO2 to induce spherule formation, and normoxia at 30°C for mycelial formation, yielding 72 cultures. The spent media were filter sterilized for volatile metabolomics analyses by headspace solid phase microextraction (HS-SPME) and two dimensional gas chromatography could with time-of-flight mass spectrometry (GC×GC-TOFMS). The metabolomes of each strain under each condition were compared using univariate and multivariate analyses.

Results

We have identified volatile metabolites that are commonly produced by both species of Coccidioides during mycelial growth, and during spherule formation. Additionally, we identified compounds that are unique to each species, but find that the variance between growth phase is greater than that of fungal species. The next steps of this work will be to collect the volatile metabolites from lung lavage specimens of uninfected and infected mice to identify which of the in vitro volatile compounds translate to in vivo infections.

Conclusions

C. posadasii and C. immitis produce volatile metabolites that may be useful in the development of a breath-based diagnostic for Valley fever lung infections.
Kim Hintzen

10:40

Hintzen K.F.H.(1,2), Smolinska A.(1), Savelkoul P.H.M.(3), Bouvy N.D.(2), van Schooten FJ.(1), Lubbers T.(2)

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(2) Department of general surgery, Maastricht University Medical Centre, Maastricht, The Netherlands
(3) Department of medical microbiology, Maastricht University Medical Centre, Maastricht, The Netherlands

Background

Abdominal sepsis is a severe condition that requires early and adequate treatment in order to improve patient outcome. With current techniques, identification of causative microorganisms in abdominal sepsis takes up to 4 days. Identification of specific volatile organic compounds (VOCs) produced by pathogens may assist in speeding up this process. The current in vitro study investigates the potential of VOC analysis as a diagnostic of pathogens involved in abdominal sepsis.

Methods

Clinical isolates of Escherichiae Coli and Enterococcus Faecalis and their antibiotic resistant subtypes, ESBL and VRE were cultured on blood agar plates. One colony was transferred into 1L culture flasks containing 100mL Trypticase Soy Broth (TSB). After overnight incubation at 37°C the headspace of the cultures was collected on stainless steel desorption tubes (1TD/Carbopack X) and analyzed by GC-tof-MS. Tree based technique was used to find pathogen specific VOCs. The final results were visualized by means of Principal Component Analysis using only pathogen-specific compounds.

Results

Analysis of the chromatograms shows a distinction between the Escherichiae Coli and he Enterococcus Faecalis based on nine distinctive VOCs with a prediction accuracy of 80%. Comparison of the pathogen specific VOCs with VOCs from their antibiotic resistant subtypes shows a distinction based on 12 VOCs for the Escherichiae Coli and ESBL (prediction accuracy 71%), while for the Enterococcus Faecalis and VRE this distinction is made based on 15 VOCs (prediction accuracy 60%). Tentative identification of VOCs reveal mostly linear- and cyclic alkenes.

Conclusions

VOC analysis demonstrates the ability to distinguish between Escherichiae Coli and Enterococcus Faecalis and is able to identify even antibiotic resistant subtypes. These in vitro results will be used to identify pathogens in an in vivo model of abdominal sepsis using exhaled breath analysis.
Ann-Christin Klemenz

11:15

Ann-Christin Klemenz (1), Juliiane Meyer (2), Katharina Ekat (2), Julia Bartels (1), Selina Traxler (1), Jochen K. Schubert (1), Günter Kamp (3), Wolfram Miekisch (1), Kirsten Peters (2)

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(2) Department of Cell Biology, University Medical Centre Rostock, Schillingallee 69, 18057 Rostock
(3) AMP-Lab GmbH, Mendelstr. 11, 48149 Münster, Germany

Background

A non-destructive and non-invasive metabolic monitoring of in vitro cultures would be a desirable alternative to classical biochemical methods, especially in stem cell research. Volatile organic compounds (VOCs) in the headspace of cell cultures may mirror adjustments of cell metabolism.

The aim of our study was to compare VOC profiles and metabolic changes of human adipose tissue-derived mesenchymal stem/stromal cells (ASC) during maintenance and lipogenic differentiation.

Methods

ASC were cultivated for three weeks under non-differentiating and adipogenically differentiating conditions. A hermetically closed, inert sampling box was used for headspace sampling. VOCs were pre-concentrated by means of needle trap micro extraction (NTME) and analyzed by GC/MS at selected time points during cultivation. The differentiation level was assessed in parallel to VOC sampling.

Results

With our non-destructive sampling system we were able to monitor cellular differentiation under standardized conditions. Emissions of 11 VOCs showed relevant concentration changes during proliferation and differentiation, respectively, and were chosen for further analysis. Concentrations of acetaldehyde, pentane and 1,3 di-tert-butylbenzene concentrations showed significant differences in the headspace of adipogenically differentiating ASC when compared to non-differentiating ASC.

Conclusions

We were able to identify VOCs, which might indicate metabolic changes during stem cell differentiation. Whether these compounds are linked to specific, enzyme-mediated processes or are the result of unspecific reactions has to be investigated. Nevertheless, VOC headspace analysis might be a suitable tool for non-destructive metabolic monitoring of in vitro cultures.
8. Detection of Mycobacterium avium ssp. paratuberculosis from native samples using VOC analysis and machine learning tools

Philipp Vitense

11:45

Vitense P (1), Küntzel A (2), Gierschner P (3), Miekisch W (3), Schubert JK (3), Köhler H (2), Reinhold P (2), Kasbohm E (1), Liebscher V (1)

(1) Institute of Mathematics and Computer Science, University of Greifswald, Greifswald, Germany;
(2) Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany;
(3) Department of Anaesthesia and Intensive Care, University Medicine Rostock, Rostock, Germany

Background

Some mycobacterial infections are associated with severe diseases. Due to their cell structure, mycobacteria are slow-growing germs, which makes diagnosis time consuming and cost-intensive. A previous study of pure mycobacteria cultures showed that the presence of growing mycobacteria can be detected via species-specific VOC profiles, even before bacterial growth is visually apparent. In the present study, this approach was used for the first time to detect infection with Mycobacterium avium ssp. paratuberculosis (MAP) from native samples.

Methods

Feces and tissue samples of healthy and MAP-infected cattle and goats were used to grow in vitro cultures. For a total of 218 cattle and 260 goat samples with varying incubation times VOCs were measured by means of NTME-GC-MS. Bacterial growth was assessed visually. Data analysis included (i) classification of presence/absence of MAP and (ii) classification of the extent of bacterial growth based on VOC data by means of random forests. This method was chosen for data analysis because of its robustness and versatility.

Results

Presence of MAP could be classified correctly for 98% of all cattle samples (feces and tissue). Classifiers for other samples reached accuracies of at least 80%. Bacterial growth could be classified with an accuracy of 84%. 39 VOCs were identified as related to MAP growth by the random forest classifier.

Conclusions

VOC analysis combined with machine learning tools enables to detect the presence of MAP and to assess the extent of bacterial growth of cultures derived from feces and tissue samples with high accuracy. Therefore, this approach is not only applicable to pure cultures of mycobacteria but also to native samples. This leads the way to possible applications for diagnostic use, which may significantly speed up diagnosis of paratuberculosis in the future.
Selina Traxler

12:05

Traxler S (1), Saß R (1), Klemenz A-C (1), Barkowsky G (2), Patenge N (2), Kreikemeyer B (2), Miekisch W (1), Schubert JK (1)

(1) Department of Anesthesiology and Intensive Care Medicine, Rostock University Medical Center, ROMBAT, Schillingallee 35 18057 Rostock, Germany

(2) Institute of Medical Microbiology, Virology and Hygiene, Rostock University Medical Center, Schillingallee 70 18057 Rostock, Germany

Background

Volatile organic compounds (VOCs) are emitted from biological cultures and are exhaled in breath. VOC trace gas analysis holds promise for complimentary information and non-invasive disease detection. Infections caused by viruses and bacteria represent a crucial problem and can lead to dangerous coinfections. This study was intended to monitor VOC headspace profiles above human cells (co)infected with different pathogens.

Methods

Human pharynx cells were infected with virus and bacteria and monitored over a defined period. For VOC analysis culture petri dishes were placed in a hermetically closed sampling system. After one hour of incubation at 37 °C, samples were taken from headspace above cultures. VOCs were pre-concentrated by sampling 20 ml headspace gas bidirectionally onto copolymer needle trap devices (NTDs). At each time point, NTDs were taken in triplicates and cell media, uninfected cells, and mono-infected cells were analyzed in the same way as controls. Pre-concentrated VOCs were thermally desorbed from NTDs, separated by gas chromatography, detected and identified by means of mass spectrometry.

Results

Significant differences in VOC profiles were detected between cell media, uninfected cells, and (co)infected cells. Emission of two VOCs increased in (co)-infected cells. These compounds enabled a differentiation of uninfected cells and (co)-infected cells. One compound showed significant higher concentrations in headspace over virus infected cells. Another compound increased significantly during bacterial mono-infection and after bacteria inoculation during the co-infection process.

Conclusions

Determining VOCs from cells (co)-infected with virus and bacteria showed promising discrimination of infections. Thus, monitoring of viral (co)-infections by means of VOC profiles holds promise for non-invasive recognition of virus and bacteria presence in vitro.
Jens Herbig
13:30
Herbig J (1), Winkler K (1), Koenemann M (1), Hartungen E (1), Sulzer P (1)
(1) IONICON Analytik, Innsbruck, Austria

Background
Proton-Transfer-Reaction – Time-of-Flight Mass Spectrometry (PTR-TOFMS) is a well-established technology for real-time quantification of trace VOCs. The technology offers many advantages for breath gas analysis: A TOFMS acquires complete spectra in a split second and is combined with ionization by proton transfer from H3O+ which is ultra-sensitive for almost all VOCs, is semi-quantitative without calibration, and is soft to suppress fragmentation. Although PTR-MS instruments equipped with Selective-Reagent-Ionization (SRI) are capable of utilizing alternative ionization modes, the majority of breath analysis studies have employed H3O+ for the reasons listed above.

In this paper we will discuss the advantages of additional ionization modes, such as NO+, O2+ and the recently added NH4+, and their advantages and disadvantages for breath analysis. While the use of NH4+ as a reagent ion is not new, we present a novel method that allows to produce NH4+ using existing hardware and gas supplies, namely from H2O and N2, thus avoiding all complications of previous implementations using corrosive ammonia in any form as a supply gas.

In addition we introduce a new concept where the same sample is analyzed in up to ten different ionization modes, consisting of various reagent ions combined with different collisional energy (E/N) levels. This yields a complete spectrum for each ionization mode with largely complementary information. To interpret this extensive amount of information we introduce a novel concept: a pattern matching algorithm in combination with a compound library is employed, which greatly enhances selectivity and specificity and allows for the separation of molecules that could not be separated in the spectrum, such as isomers.
Background
Today’s society puts more and more importance on personal health and wellbeing. People want to take care of their health and optimize their personal performance through weight management, healthy eating and the practicing of various sports. Unfortunately, there is a lack of simple and adequate means of evaluating the current status of one's health and monitoring the effects of actions undertaken for our wellbeing.

Methods
At MICROSENS we have created a breath analysis system, named "Breath Intelligence", which is based on gas chromatography similar to medical breath analysis systems but on a simplified, smaller and thus faster scale. At its heart it combines a highly sensitive semiconductor gas sensor with a micro-fabricated gas separation column to form a small measurement unit. The measurement unit is connected to a pneumatic circuit with miniature valves and a small pump. Together these elements form an easy-to-use handheld system for breath analysis which enables us to scan for multiple breath compounds with one single system. From a single breath sample we can obtain information on a range of breath compounds and thus have a more holistic view of a person’s metabolism.

Results
The system was used for halitosis monitoring and the results were compared to those of other systems and methods. The study showed that “Breath Intelligence” yields results comparable to current commercial systems aimed at the specific detection of VSC (e.g. OralChroma). More recently the system was employed to monitor acetone in the breath of healthy individuals without any specific diet or exercise routine. Based on previous research, acetone levels should be around 0.5 – 2ppm, which was also measured in the test.

Conclusions
“Breath Intelligence” is a handheld and easy-to-use system for measuring metabolic markers in breath that will give you the information you need to easily adjust your daily routine and in consequence make the most of your training and nutrition.
12. Further Evaluation of a Standardized Breath Sampling Device for Off-line Exhaled Breath Analysis

Sean Harshman

Harshman SW (1), Pitsch RL (2), Davidson CN (3), Scott AM (3), Strayer KE (1), Hill EM (1), Smith ZK (1), Brothers MC (1), Schaeublin NM (1), Slusher GM (1), Meoli SD (1), and Martin JA (3)

(1) UES Inc.,
(2) The Henry M. Jackson Foundation for the Advancement of Military Medicine,
(3) Air Force Research Laboratory; 711th Human Performance Wing/RHXBC, Wright- Patterson AFB, OH, USA

Background

Exhaled breath bags have been the standard method for off-line breath collection. However recently, the Respiration Collector for In Vitro Analysis (ReCIVA) was developed for versatile exhaled breath collection directly onto adsorptive material. The ReCIVA is designed to eliminate sources of variability associated with off-line exhaled breath collection. While potentially beneficial, very little has been done to characterize the overall performance of the ReCIVA breath sampler.

Methods

All participants were fasting (≥1h) males within our facility. Collections on the ReCIVA were performed, as described by the manufacturer, at 200mL/min for 550mL of lower airway breath except where manual calibrated flow rates were applied. All exhaled breath bags were collected for end tidal breath in 1L ALTEF bags using our established collection protocol. Breath volatiles were concentrated utilizing both Tenax TA and Carbograph/Tenax 5TD thermal desorption tubes. For multiple ReCIVA comparisons, ReCIVA serial #33 and #65 were used. For breathing rate determinations the Breathe+ iPhone app was used to guide participants breathing rates to either high, 15 breaths/min, or low, 7.5 breaths/min. All thermal desorption tubes were analyzed by TD-GC-MS using 70eV EI. Isoprene was quantified from samples using calibration curves determined from a custom isoprene canister (1.10ppm).

Results

The results demonstrate using a custom "in-house" flow calibration on the ReCIVA yields consistent isoprene results among both ReCIVA banks (p>0.3131), TD tube types (p=0.3824 between Tenax TA and 5TD tubes), and between the ReCIVA and standard exhaled breath bags (p=0.1534). Furthermore, comparisons among ReCIVA units #33 and #65 using the "in-house" flow calibrations show comparable isoprene results between both units (p=0.1441). Finally, isoprene values from controlled rate breath show no significant difference between the rates (p=0.6666). However, for experiments comparing ReCIVA units and breathing rates, a significant difference among the Tenax TA and 5TD tubes was observed (p<0.0053).

Conclusions

The results suggest the ReCIVA can provide reliable exhaled data independent of the ReCIVA device and breathing rate. These data support the use of the ReCIVA for widespread breath collections.
Florian Schmidt

Ghorbani R (1), Muala A (2), Blomberg A (2), and Schmidt FM (1).

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(2) Department of Public Health and Clinical Medicine, Umeå University, SE-90187 Umeå, Sweden

Background

Smoke from combustion of biomass fuels is a major risk factor for respiratory disease. Recent findings show that short-term exposure to PAH rich wood smoke from incomplete combustion induces cell death and DNA damage in healthy subjects, but only minor inflammatory responses. The aim of this study is to investigate exhaled breath carbon monoxide (eCO) in response to wood smoke exposure.

Methods

A 2 h double-blind exposure study with air and wood smoke (400 μg/m³ PM0.4, 10 ppm CO) is conducted with 14 healthy non-smokers. For two subjects, the elimination kinetics after exposure to 10 ppm CO and wood smoke is measured and compared to the Coburn-Forster-Kane model. Real-time eCO detection is coupled to pulmonary gas exchange modeling to extract end-tidal CO, lung diffusion capacity and airway tissue CO concentration from single exhalations. The eCO profiles are measured using mid-IR laser absorption spectroscopy (9 ppb detection limit and 2 ppb precision at 0.1 s acquisition time). A trumpet model with axial diffusion (TMAD) is used to simulated the CO gas exchange and fit the eCO profiles.

Results

After exposure to 10 ppm CO, end-tidal CO and airway tissue CO are increased, while the lung diffusing capacity remains unchanged. A similar response is observed after exposure to wood smoke, with end-tidal CO increased by a factor of 3-5, but the lung diffusing capacity is slightly decreased. The CO elimination curve follows the CO half-life of about 5 hours.

Conclusions

The significant increase in end-tidal CO after exposure to wood smoke can solely be explained by the uptake and elimination of the CO in the smoke. This suggests that there is no inflammatory response measurable with eCO in healthy non-smokers. The effect of exposure to wood smoke particles on the lung diffusion capacity measured with extended eCO analysis should be further investigated.
Makoto Sawano

15:55

Sawano M (1), Fukushima K (1), Inoue K (2)
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Background: Locating survivors trapped in rubble is a top priority in post-disaster search and rescue operations. A CO2 beacon that tracks survivor’s CO2 exhalation has been developed to support the operations. However, at disaster sites, there are many sources of CO2 such as fires, sodas, rescue workers and animals that interfere with the CO2 beacon and make it less useful. We developed a novel 13CO2 beacon, based on the fact that the increase in the atmospheric CO2 isotope ratio (13CO2/12CO2) is only caused by respiration of survivors who ingested 13C-rich compounds. This study evaluates the efficacy of 13CO2 beacon to locate a survivor in a simulated disaster site.

Methods: A subject is placed in a simulated disaster site after ingestion of 1g 13C(99%)-glucose. Then, atmosphere was sampled at 1m to 5m distance, intermittently until 12hrs. The samples were introduced into GC-MS after preconcentration using SPME(Carboxen/PDMS). The GC-MS equipped with Agilent HP-LOT Q column provided single-ion chromatogram for 44m/z (12CO2) and 45m/z (13CO2), which allowed precise estimation of CO2 isotope ratios from their AUCs.

Results: Δ13C (increase of CO2 isotope ratio from the background) of the samples showed significant increase after 30min (46100 [∂0/00] at 1m, 800 at 5m), peaked at 3hrs (146200 at 1m, 19300 at 5m) and gradually decreased until 12hrs, but did not recover the baseline. At all time, Δ13C showed reverse proportion with square of the distance, regardless of the obstacles which simulated the rubbles.

Conclusions: The results indicated that 13CO2 beacon can efficiently locate the survivor who ingested 13C-glucose by mapping atmospheric CO2 isotope ratios. 13CO2 beacon is valid for 12hrs or longer following the ingestion and is not interfered by rubbles or environmental CO2 sources. The study is the first to report application of 13C breath tests to disaster relief, and precise estimation of CO2 isotope ratio in atmospheric CO2 with minute concentration of 300 to 500.
Identification of a large set of volatile organic compounds characteristic for cystic fibrosis in children

Tobias Bruderer

Bruderer Tobias (1,2), Weber R (1), Haas Naemi (1), Baghdasaryan A (1,3), Inci D (1), Micic S (1), Perkins N (4), Bähler P (1), Spinas R (1), Zenobi R (3), Möller A (1)

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(2) ETH Zurich, Department of Chemistry and Applied Bioscience, Zurich, Switzerland,
(3) Joint Medical Center Arabkir, Division of Pulmonology, Yerevan, Armenia,
(4) Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Switzerland.

Background

Early pulmonary infection and inflammation result in irreversible lung damage and are major contributors to Cystic Fibrosis (CF) related morbidity. An easy to apply and non-invasive assessment for the timely detection of disease-associated complications would be of high value. We aimed to detect disease specific exhaled VOCs in children by real-time secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS).

Methods

A total of 101 children, aged 4-18 years (CF = 52; healthy controls = 49) and comparable for sex, body mass index and lung function were included in this case-control study. Online breath analysis was done with SESI-HRMS. Carefully pre-processed MS data was used for biomarker detection and classification. 171 m/z features were significantly different in children with CF (FDR adjusted p-value of 0.05). Compound identification was done with SESI-HRMS/MS.

Results

We were able to annotate 95% of the 171 CF features (m/z features) with a putative molecular formula based on a correlation matrix to assign common adducts, clusters and losses. This was in contrast to our previous CF study where only 37% of 49 features could be assigned with an unambiguous formula which was probably most likely due to the formation of non-generic fragments with the previous SESI-MS instrumentation. Our previously reported identification workflow based on exhaled breath condensates showed limited use for the present study. Therefore, we present first results from an on-line compound identification approach with a pre-separation step. We could identify molecules from several metabolic classes including highly oxidized compounds, carnitines and long-chain fatty acids and their oxidation products. We are currently investigating the involved metabolic pathways in more detail.

Conclusions

We have detected a large set of exhaled molecules characteristic for CF from several metabolic classes including highly oxidized compounds, carnitines, fatty acids and their oxidation products.
Background

Pulmonary nodules are being detected with increasing frequency because of the increased use of chest computed tomography (CT). Every noncalcified, non-fat-containing nodule must be regarded as potentially malignant and accurately predicting malignant nodules is not straightforward. The “wait and watch” strategy may be worrying. In view of this, we aim to find potential biomarkers to identify lung adenocarcinoma and pulmonary granuloma.

Methods

In this study, 23 patients undergoing elective thoracoscopic lobectomy were involved. After pathology diagnoses, one patient with metastatic tumor and another with lymphoma were excluded. Plasma and urine samples were collected simultaneously at four time points in all of these patients: time point before anesthesia(T1), 5min after incubation(T2), 5min after tumour removal(T3), and 5min after extubation(T4). All plasma and urine samples were analyzed with a HS-GC-IMS instrument (FlavourSpec®) from G.A.S.(Ge Gesellschaft fur Analytische Sensorysteme GmbH, Dortmund, Germany), equipped with an autosampler unit(CTC Analytics AG, Zwingen, Switzerland) that can be directly sampled from the headspace by using a 1mL syringe. GC was fitted with a 15m gas chromatographic column (FS-SE-54-CB-1, ID: 0.53 mm) to separate volatile components and coupled to IMS. The data was acquired in positive mode and analyzed using LAV software (version 2.2.1) from G.A.S. Principal component analysis (PCA) and partial least-squares discriminant analysis (PLSDA) were performed as the statistical methods to process the final data.

Results

Several VOCs were considered as potential different markers in 14 lung adenocarcinoma compared to 7 pulmonary granuloma patients, both in plasma and urine. In plasma, 1-propanol, cyclohexanone, benzaldehyde, acetophenone, nonanal, heptanal and octanal were identified, while other different 9 compounds were going to be characterized after IMS database expanding. In urine, 1-propanol, benzaldehyde, nonanal, heptanal, 1-hexanol, 2-octanone, cis-3-hexenol were considered to be different, while other 7 compounds were under characterized. A separation trend was showed in a two-dimensional PCA score plot. The PLSDA score plot demonstrated an optimal separation trend between the lung adenocarcinoma patients and granuloma patients. We also found the changes of VOCs concentration during anesthesia both in plasma and urine according to plugin gallery result of two patients. Concentrations of 1-butanol, cyclohexanone, compound 17#,24#,29# in plasma increased after anesthesia beginning, and decreased or disappeared after anesthesia finished. In urine, concentrations of cyclohexanone, acetophenone, 2-ethyl-1-hexanol and several uncharacterized VOCs had the similar
THE TURING EXHIBITION SPACE

changes as in plasma, while ethyl propanoate, 2-pentanone concentrations decreased after anesthesia beginning to some extent.

Conclusions

Using HS-GC-IMS, several VOCs in blood and urine were found as potential biomarkers to identify lung adenocarcinoma from pulmonary granulom patients, which may be helpful for early detection and identification of lung adenocarcinoma. In addition, the changes of VOCs in blood and urine during perioperative period may be related to inhibition of oxidative stress response or medicine metabolites.
<table>
<thead>
<tr>
<th>Start</th>
<th>Finish</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:50</td>
<td>10:20</td>
<td>Direct Detection of Sub-ppbv Level of breath n- alkanes by Photoelectron Induced O2+ Cation Chemical Ionization Mass Spectrometry</td>
<td>Lei Hua</td>
</tr>
<tr>
<td>10:20</td>
<td>10:40</td>
<td>Using labelled internal standards to improve needle trap micro-extraction technique prior to gas chromatography/mass spectrometry</td>
<td>Tommaso Lomonaco</td>
</tr>
<tr>
<td>10:40</td>
<td>11:00</td>
<td>Development Of An IMS-Based Method For Passenger Control At Airports: A Proof-Of-Concept Study</td>
<td>Isabel Steppert</td>
</tr>
<tr>
<td>11:15</td>
<td>11:45</td>
<td>Automated Thermal Desorption (TD)-SIFT-MS: A New Paradigm for Breath Analysis</td>
<td>Nathan Hawkins</td>
</tr>
<tr>
<td>11:45</td>
<td>12:05</td>
<td>Taking Soft Chemical Ionisation Mass Spectrometry techniques on the “Walk of the World” a breath taking adventure</td>
<td>Ben Henderson</td>
</tr>
<tr>
<td>12:05</td>
<td>12:25</td>
<td>Selective and Sensitive Measurement of Trace Exhaled HCN by Acetone-Assisted Negative Photoionization Time-of-flight Mass Spectrometry</td>
<td>Haiyang Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETNO as a biomarker for investigating the effect of different OLV strategies on lung injury and inflammation response</td>
<td>Yang Lyu</td>
</tr>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Stability of FENO50 in a COPD cohort in Sweden over 2-year follow-up</td>
<td>Marieann Högman</td>
</tr>
<tr>
<td>14:35</td>
<td>14:55</td>
<td>A proposed data standard for breath sample data and metadata</td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td>15:55</td>
<td>Combining field-asymmetric ion mobility spectrometry (FAIMS), Infrared (IR) and luminescence sensing (LS) for artificial breath analysis</td>
<td>Tamina Hagemann</td>
</tr>
<tr>
<td>15:55</td>
<td>16:15</td>
<td>Collection of breath samples for offline ‘breathomics’ mass spectrometry analysis using the ReCIVA® device in patients with acute breathlessness: a feasibility study</td>
<td>Karl Holden</td>
</tr>
<tr>
<td>16:15</td>
<td>16:35</td>
<td>Real-time breath analysis with SESI-HRMS, confounding factors &amp; standardization strategies to initiate multi-center studies</td>
<td>Guillermo (William) Vidal de Miguel</td>
</tr>
</tbody>
</table>
**HEADLINE:** Direct Detection Of Sub-ppbv Level Of Breath N-Alkanes By Photoelectron Induced O2+ Cation Chemical Ionization Mass Spectrometry

Lei Hua

09:50

Lei Hua (1), Yan Wang (1), Enyou Li (2), Lei Guo (2), Yang Li (1), Haiyang Li (1)

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(2) Department of Anesthesiology, The First Affiliated Hospital of Harbin Medical University, Harbin, China.

**Background**

Alkanes are a kind of significant product in human metabolic processes. However, online and real-time measurement of saturated hydrocarbons, especially small n-alkanes, using direct mass spectrometry, remains a great challenge due to their low basicity and fewer ionizable functional groups.

**Methods**

A high-pressure photoionization induced O2+ cation chemical ionization (HPPI-OCI) source was developed and coupled with a time-of-flight mass spectrometer. An efficient online pretreatment system was developed for eliminating the interference of humidity and carbonyl compounds. High-intensity O2+ reactant ion could be generated by photoelectron ionization for efficient ionization of n-alkanes.

**Results**

The HPPI-OCI mass spectrum pattern of C3-C6 n-alkanes differed a lot at different ion source pressure. The quasi-molecular ions [M-H]+ were gradually dominating and the fragmentation of n-alkanes by O2+CI was depressed while the ion source pressure was elevated from 88 to 1080 Pa, with more than three orders of magnitude improvement in signal intensity. The achieved LODs (S/N=3) were down to 0.07-0.14 ppbv for C3-C6 n-alkanes in one minute. The HPPI-OCI TOFMS was successfully used in the analysis of exhaled small n-alkanes of healthy smokers and nonsmokers as well as the concentration variation of exhaled small n-alkanes after alcohol consumption.

**Conclusions**

This work not only provides new insights for controlling the chemical ionization process by adjusting the ion source pressures but also prompts an innovative and high-performance direct MS technique for small n-alkanes analysis. It is likely that the HPPI-OCI MS will be a powerful tool in the field of high-throughput clinical diagnostics.
Using Labelled Internal Standards To Improve The Analytical Performance Of Breath Analysis By Needle Trap Micro-Extraction Gas Chromatography-Mass Spectrometry (NTME-GC-MS)

Tommaso Lomonaco
10:20

Biagini D (1), Lomonaco T (1), Ghimenti S (1), Onor M (2), Bellagambi FG (1), Salvo P (3), Di Francesco F (1), and Fuoco R (1).

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Background

When working with humid gaseous samples, the amount of water vapor collected in a needle trap along with VOCs may vary from sample to sample and decrease during the storage. This has a major impact on analyte desorption efficiency and recovery. We propose the addition of a labeled internal standards (ISs) to virtually cancel the effect due to humidity variability on the analytical performance of NTME combined with GC-MS.

Methods

Triple-bed (Divinylbenzene/Carbopack X/Carboxen 1000) and single-bed (Tenax GR) needles were tested with standard gaseous mixtures prepared at different relative humidity (RH) levels (85, 50 and 10%). The standard mixtures contained 25 analytes representative of breath and ambient air constituents, including hydrocarbons, ketones, aldehydes, aromatics, and sulfurs, in the concentration range 0.1-700 ppbv.

Results

The tested needles showed different behaviours, as recovery was independent of humidity for single-beds, whereas a low recovery (10-20%) was observed when triple-beds trapped very volatile compounds (e.g. pentane and ethanol) at humidity level as low as 10% RH. Triple-beds showed an almost quantitative recovery (>90%) of all the analytes at 50 and 85% RH. The addition of 6D-acetone and 8D-toluene to the sorbent material before gas sampling and the normalization of raw data almost nullified this effect, thereby lowering the variations of analyte recovery at different RH levels down to 20%. After normalization, the inter- and intra-day method precision were halved to 5% and 10% in the case of single-beds, respectively, and to 15% and 20% with three-beds.

Conclusions

The addition of ISs to the sorbent in the needle trap helps to keep under control data reproducibility and improves data reliability of NTME-GC-MS for the determination of VOCs in breath and ambient air samples.
19. Development Of An IMS-Based Method For Passenger Control At Airports: A Proof-Of-Concept Study

Isabel Steppert

10:40

Steppert I (1), Bangen LM (1), Schönfelder J (1,2) and Kuhlmeier D (1,2)

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(2) Fraunhofer Project Hub "Microelectronic and Optical Systems for Biomedicine" MEOS, Erfurt, Germany

Background

With the increase in air traffic, infectious diseases spread faster around the world and thus are of global concern. One strategy to reduce this spreading is the screening of passengers for infections. Nevertheless, the actual screening methods like questionnaires and fever measurements are error-prone and do not allow an identification of pathogens. This project aims to develop a non-invasive ion mobility spectrometry (IMS) based method for the identification of infectious pathogens from breath based on their distinct volatile organic compound (VOC) fingerprints. IMS is fast, easy-to-use and already employed for explosive and drug screening at airports.

Methods

Before carrying out the analysis of human exhaled breath, in-vitro studies on bacterial cultures have been executed to verify the IMS method. Several bacterial species were investigated in culture media by multicapillary column (MCC) coupled IMS. The VOCs emitted by bacteria in the headspace of cultures were drawn via tube into the analysis device using an internal pump, were ionized by a Tritium source and measured by MCC-IMS. For the differentiation of bacteria, the VOC derived peaks in IMS spectra were analyzed using a cluster analysis-based software and statistical methods.

Results

Using a cluster analysis, several hundred different clusters representing VOCs were derived. Within these, significant different VOCs could be found for all bacteria by using statistical methods. For each bacterial species, at least one significant VOC peak was found which is distinct for one pathogen and not present in other bacterial species or controls. Sensitivity and specificity were calculated for each cluster. Several clusters obtain a sensitivity and specificity of 80% or above.

Conclusions

MCC-IMS is suitable for a rapid identification of bacteria based on their distinct VOCs in culture. One MCC-IMS analysis takes only 4-5 minutes. We showed that in-vitro differentiation between different bacterial species and controls (culture medium) by a number of VOCs was possible, if tested less than 2 hours after inoculation. Next step is to perform a clinical study using exhaled breath from infected and healthy individuals.
Automated Thermal Desorption (TD)-SIFT-MS: A New Paradigm for Breath Analysis

Nathan Hawkins

11:15

Hawkins N (1), Bell KJM (2), Padayachee D (2), Langford VS (2)

(1) Anatune Ltd, Cambridge, United Kingdom
(2) Syft Technologies Ltd, Christchurch, New Zealand

Background

The recently released combination of automated thermal desorption with real-time SIFT-MS has opened up a realm of new rapid analysis possibilities, with breath profiling being one of the most promising applications. Some initial evaluations were carried out to investigate the potential of rapid breath profiling for a variety of oxygenated and reduced sulfur compounds.

Methods

A Voice200ultra (Syft Technologies Ltd, Christchurch, New Zealand), coupled with a MPS robotic pro autosampler, TD 3.5+ thermal desorption unit and CIS4 transfer line (GERSTEL, Mülheim an der Ruhr, Germany) were used to analyse breath volatiles loaded onto TD tubes. The Voice200ultra was calibrated for reduced sulfur compounds using a gas standard and assessed for linearity and repeatability. Air and breath samples were then spiked with reduced sulfur compounds and loaded onto both Tenax TA and Tenax TA/Carbograph 5TD tubes, and desorption profiles and desorbed masses were compared as a function of the adsorbent material. Finally, the breath of volunteers who had gone without using oral hygiene overnight (for a period of at least 12 h), was loaded onto TD tubes and analysed.

Results

Excellent linearity and repeatability were obtained for most of the reduced sulfur gas standard compounds loaded on the TD tubes. Real-time desorption profiles showed multiple desorption peaks, probably related to the different adsorption sites in the TD tube materials. Differences in adsorbent materials were more pronounced for methyl mercaptan and ethyl mercaptan, while better agreement was obtained for compounds such as dimethyl sulfide and isoprene. In this initial evaluation, each tube was analysed in less than 7 minutes.

Conclusions

Both accuracy and precision of calibrated TD-SIFT-MS were demonstrated in this evaluation, along with the excellent speed of analysis. The ability to do rapid analysis of TD tubes will allow for larger sample throughput for testing laboratories and rapid clinical pre-screening, yielding both time- and cost- savings.
Ben Henderson

11:45

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(2) Dept. of Marine and Atmospheric Research, Utrecht University, Utrecht, Netherlands

Background

The 4 Days March in Nijmegen is a unique event known as the “Walk of the World” which attracts up to 45,000 people each year. Participants walk either 30, 40 or 50 km per day for 4 days. This event offers an exciting opportunity to demonstrate the potential of using soft chemical ionisation mass spectrometry (SCIMS) technologies, such as Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) and Gas Chromatography Ion Mobility Spectrometry (GC-IMS) outside of a traditional laboratory environment for online longitudinal untargeted breath measurements.

Methods

100 participants were sampled online via direct exhalation through a commercial breath sampler equipped with a CO2 sensor (®Loccioni) coupled to the inlet line of the PTR-ToF-MS. A trigger was connected between the PTR-ToF-MS and the breath sampler with a threshold set of 4% CO2 in order to assist with the data processing. A sub-group of 45 out of the 100 participants were additionally sampled offline in a 3 L Tedlar bag for later analysis on GC-IMS, since the time available to sample an individual needed to be less than 2 minutes. The participants were sampled prior to the start of the event as baseline measurements, then before and after walking on the first 3 days of the march.

Results

On the PTR-ToF-MS over 600 m/z values were measured. Using Analysis of Variance-Simultaneous Component Analysis (ASCA), the breath profiles could be clearly separated based on the day of walking and before and after walking. For the GC-IMS measurements, 30 peaks were selected from the chromatograms that could be used to discriminate breath profiles based on the effect of walking over the 4 days.

Conclusions

SCIMS technologies are suitable for use in large scale breath analysis projects in non-traditional laboratory environments, allowing discovery of potential metabolites for physical activity related processes. Data processing methods have been successfully developed which allow further statistical analysis. Through use of statistical tools, the effect of strenuous exercise on the complete VOC profile of participant’s breath is revealed.
Selective and Sensitive Measurement of Trace Exhaled HCN by Acetone-Assisted Negative Photoionization Time-of-flight Mass Spectrometry

Haiyang Li
12:05

Haiyang Li (1), Yuanyuan Xie (1), Enyou Li (2), Yiping Liu (2), Lei Guo (2), Lei Hua (1)
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(2) Department of Anesthesiology, The First Affiliated Hospital of Harbin Medical University, Harbin, China.

Background
Exhaled hydrogen cyanide (HCN) is one of the most promising breath markers for respiratory diseases such as cystic fibrosis (CF) mainly caused by pseudomonas aeruginosa (PA) airway infection. Its concentration profile for exhalation can provide useful information for medical disease diagnosis and therapeutic procedures. However, the high-level moisture and carbon dioxide (CO2) in exhaled gas always lead to big quantification complexity, poor selectivity and sensitivity for existing analytical techniques.

Methods
Acetone-assisted negative photoionization (AANP) with CN- (m/z 26) as the target ion based on a vacuum ultraviolet (VUV) lamp in a time-of-flight mass spectrometer (AANP-TOFMS) was firstly proposed for online measurement of trace HCN in human breath. In-source collision-induced dissociation (CID) was adopted for signal improvement of the target ion CN-. Matrix influences in the breath including moisture and CO2 were investigated, respectively, and a Nafion tube was used for online dehumidification of breath samples.

Results
The signal response of the target ion CN- was improved by about 24-fold with CID. The matrix-adapted calibration in the concentration range of 0.5~50 ppbv with satisfactory dynamic linearity and repeatability was obtained. The lowest detectable HCN concentration at sub-ppbv was achieved in consideration of matrix effects of moisture and CO2. The method was successfully applied for determination of human mouth- and nose-exhaled HCN, and optimum HCN data were achieved to assess systematic HCN levels for healthy individuals.

Conclusions
Together with in-source CID and matrix-adapted calibration, AANP-TOFMS provides a robust and attractive tool for online measurement of exhaled HCN for noninvasive clinical diagnosis such as CF disease and cyanide poisoning in the following work.
ETNO As A Biomarker For Investigating The Effect Of Different OLV Strategies On Lung Injury And Inflammation Response.

Yang Lyu

14:15

Yang Lyu(1), Xin Pi(1), Dongchun Wang(1), Lei Guo(1), Desheng Liu(1), Lin Cui, Meng Li(1), Yinhua Cui(1), Zhongzhi Qiu(1), Jinghui Shi(1) and Enyou Li(1)

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Background

One-lung ventilation (OLV) aggravates alveolar damage and inflammation response in the lung. The evaluation indicators of lung injury caused by OLV are not perfect. End-tidal fraction of nitric oxide (ETNO) continuously collected during ventilation may be a new and non-invasive inflammatory marker of lung injury to investigate the effect of different OLV strategies.

Methods

A total of 59 patients undergoing thoracic surgery were included and randomized into two groups. These patients had the same parameters during two-lung ventilation, but during OLV, the High Volume group was set at a tidal volume (VT)=8 ml/kg PBW and a positive end-expiratory pressure (PEEP)=5 cmH2O, while the Low Volume group was set at a VT=5 ml/kg PBW and a PEEP=5 cmH2O with recruitment every 30 min. ETNO was acquired at the points of induction, OLV 0 min, OLV 15 min, OLV 30 min, OLV 1 h and immediately at two-lung re-ventilation. We also obtained traditional evaluation indices at the same points.

Results

ETNO did not differ significantly between groups at baseline. When the patients suffered OLV, compared with the Low Volume group, ETNO in the High Volume group significantly decreased at all points (P<0.001), and the expression of endothelial NO synthase in plasma decreased but lagged for several quarters. There was almost no change in traditional inflammatory factor in plasma.

Conclusions

Compared with traditional inflammatory factor, ETNO can be a new, rapid, convenient and accurate inflammatory marker for investigating the effects of different OLV strategies in early-phase lung injury and pro-inflammation response.
Marieann Högman

14:35

Högman M (1), Lisspers K (2), Ställberg B (2), Bröms K (2), Janson C (1), Malinovschi A (3).

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Background
The main goal of COPD therapy is to control symptoms, reduce inflammation and preserve lung function. The aim was to investigate the stability of the inflammatory marker FENO over two years in COPD subjects during stable conditions.

Methods
Subjects from the Swedish Tools for Identifying Exacerbations (TIE)-study with spirometry-verified COPD diagnosis were included. FENO50 was measured before spirometry. Data are from inclusion visit, 1 and 2 years later and presented as median (25th and 75th percentile) or mean ± SD.

Results
In the subjects that participated in all visits (n=352) the FENO50 at inclusion was 13 (9, 21) ppb, at 1-year follow-up 14 (9, 23) ppb and at 2-year follow-up 13 (8, 21) ppb, p=0.14. A Bland Altman analysis of the inclusion visit and first year follow-up showed a mean difference of -0.6 ppb (95%CI of the difference of -27, 26 ppb). Corresponding result for first year follow-up and the 2-year follow-up is 0.6 (95%CI of the difference of -26, 27 ppb) and for the inclusion to the 2 years follow up gives a mean difference of -0.40 ppb (95%CI of the difference of -30, 29 ppb). There was no trend in the Bland Altman plot (p=0.08).

Lung function was performed at all visits and at inclusion the FEV1-% predicted was 58 ± 16, at 1-year follow-up 58 ± 17 and at 2-year follow-up 57 ± 17 % predicted, p=0.031. There was positive correlation between FEV1 expressed as % predicted and FENO50 at inclusion (rho=0.13, p=0.012), at 1-year follow-up (rho=0.12, p=0.033) and at 2-year follow-up (rho=0.18, p<0.001).

Conclusion
The FENO50 levels were stable over the 2-year follow-up period in COPD subjects investigated in stable condition, which is positive finding of an inflammatory marker. There was a statistically significant association between low FENO50 and low FEV1 at all time points. Further studies are needed regarding the clinical use of FENO50 in COPD.
Headline: A proposed data standard for breath sample data and metadata

Bo Zhao

15:30

Zhao B* (1), Salman D (3), Wilde MJ (2), Cordell R (2), Bryant L (2), Ruszkiewicz D (3), Singapuri A (1), Ibrahim W (1), Brightling C (1), Siddiqui S (1), Thomas CLP* (3), Free R* (1)

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(2) Department of Chemistry, University of Leicester, Leicester, UK
(3) Centre for Analytical Science, Department of Chemistry, Loughborough University, Loughborough, UK

Background

Data management in breath research is underdeveloped. While general metabolomics data standards exist, these do not include detailed individual sample provenance and quality assurance: something which is particularly important in the breathomics field. To facilitate quality control and data sharing, we created a new data standard and informatics tools which included sample specific metadata as a key component.

Methods

We extended an existing data standard (ISATAB) with support for sample specific metadata. Then developed informatics tools to facilitate straightforward data standard creation, management, report generation and sample data sharing. Our proposed data standard uses re-usable flexible scaffolds and templates to handle different equipment and processes and could be adopted by any study requiring consistent metadata collection. It includes support for metadata describing individual sample processing, provenance and quality and production of individual data header reports.

Results

To demonstrate the applicability of the proposed standard we used breath sample data collected during the East Midlands Breathomics Pathology Node project. Collaborating researchers used an example scaffold to integrate their existing data files into a template, with which they produced outputs including a data archive (containing data and metadata), and ‘data header’ PDF reports pertaining to individual samples were produced.

Conclusions

The proposed data standard and informatics tool simplify the metadata collection and reporting process. Easily shareable PDF-based data headers allow researchers to understand each step the sample has gone through, as well as repeat relevant analytical processes. Furthermore, the ISATAB-compatible data archive supports uploading to existing repositories, while also including individual sample meta-data.
Combining field-asymmetric ion mobility spectrometry (FAIMS), Infrared (IR) and luminescence sensing (LS) for artificial breath analysis

Tamina Hagemann

15:55


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Background

Currently, detection of carbon dioxide and oxygen by Fourier transform infrared (FTIR) spectroscopy based on substrate-integrated hollow waveguides (iHWGs) and luminescence sensing (LS), respectively, is enabled in the mouse intensive care unit at the Institute of Anesthesiologic Pathophysiology and Method Development (IAPMD). In order to obtain deeper metabolic information, the presented study focused on additionally enabling the detection of volatile organic compounds (VOCs) in breath.

Methods

A hybrid analytical platform comprising field-asymmetric ion mobility spectrometry (FAIMS), iHWG-based FTIR spectroscopy and LS was built and tested by simple, artificial breath samples containing nitrogen, oxygen (20%), carbon dioxide (3-5%) and acetone (0-23 ppb).

Results [1]

Feasibility of integration of FAIMS, iHWG-FTIR and LS into a single analytical setup suitable for online monitoring was demonstrated. Quantification of acetone, carbon dioxide and oxygen was enabled in the respectively breath-relevant concentration range without any sample preseparation. Orthogonality of the three methods was shown.

Conclusions

The presented hybrid analytical technique is promising for comprehensive breath analysis. However, more complex artificial breath samples (multiple VOCs, humidity), as well as real breath samples need to be measured Furthermore, enabling preseparation and setup adaptation to mouse breath will be necessary.

Collection Of Breath Samples For Offline ‘Breathomics’ Mass Spectrometry Analysis Using The ReCIVA® Device In Patients With Acute Breathlessness: A Feasibility Study

Karl Holden

16:15
Holden KA (1)*, Ibrahim W (1)*, McNally T (1), Salman D (2), Beardsmore C(1), Wilde M (3), Cordell R (3), Bryant L (3), Monks PS(3), Brighting C (1), Thomas CLP(2), Siddiqui S(1)*, Gaillard EA(1)*

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(2) Centre for Analytical Science, Department of Chemistry, Loughborough University, Loughborough, UK
(3) Department of Chemistry, University of Leicester, Leicester, UK

Background
Investigating the underlying cause of acute breathlessness and also underlying inflammatory processes in respiratory disease in both adults and children is both technically and ethically challenging. Clinical assessment and blood biomarkers have limitations as discriminators. Being able to identify and validate non-invasive, novel methods such as analysing the metabolic content of exhaled breath would be a huge clinical advance. Within this advancing field of breathomics both online and offline methods of collecting breath samples have been assessed. The ReCIVA breath sampling device allows breath samples to be collected directly into sorbent tubes for analysis of exhaled volatile organic compounds. We aimed to assess the feasibility of using this in acutely breathless patients.

Methods
Adults attending with acute breathlessness and children aged 5-16 years attending our hospital with acute asthma or chronic, stable asthma as well as controls were recruited. Breath samples were collected into thermosorbent tubes using the ReCIVA device and sent for analysis by means of gas-chromatography x gas-chromatography mass spectrometry.

Results
We present data for 65 adults recruited with pneumonia (n=15), heart failure (n=14), acute asthma (n=1), acute COPD (n=21) and controls (n=14) and 61 children recruited with chronic asthma (n=23), acute asthma (n=29) and controls (n=9). We found that 98.4% of adults and 75.4% of children were able to provide the full target breath sample using the ReCIVA device. At an early stage we discovered irregularities in signals detected which was attributed to a change in the manufacturer of gloves personnel were using. NASA measurements of perceived task workload were available in the adult population with mean values of 3.37 for effort, 2.34 for frustration, 3.8 for mental demand, 2.8 for performance, 3.9 for physical demand an 2.8 for temporal demand.

Conclusions
This feasibility study demonstrates it is possible to collect breath samples from both adults and children with acute breathlessness/asthma, chronic asthma and controls for breathomics analysis using the ReCIVA device. Strict study protocols are required to reduce irregularities in signals detected. Further work should involve a) recruiting patients in a large-scale multi-centre study to obtain large numbers of breath samples and b) development of a workflow to analyse breath samples and create breath profiles.
Guillermo (William) Vidal de Miguel

16:35

Vidal de Miguel, G (1,2), Macia, M (1), Barreiro P (1), Singh (3), Sinues P (3)
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(2) Deep Breath Initiative AZ, Zug, Switzerland;
(3) Biomedical Engineering, Basel University, Basel, Switzerland

Background

The introduction of breath analysis into routine clinical practice remains a challenge, mostly because of insufficient statistical significance and uncontrolled confounding factors.

Methods

We combined a real-time SESI-HRMS breath analyzer with a systems that measures exhaled flow, volume and CO2, designed to operate in the range of flows and pressures of the analyzer and to guide the exhalation maneuver, and measures breath of healthy individuals. For a set of oxidative stress biomarkers, we studied the different sources of confounding factors, and define strategies to improve repeatability of the tests. The ultimate goal of these studies is to provide standardized tools and protocols to enable further developments such as multi-center clinical studies.

Results

a) Instrument Coefficient of Variation: 2.3%, evaluated with β-pinene from gas standard generator.

b) Average number of peaks detected: 8000, with dynamic range of 10E5, most peaks in 100-300 Da range. To reduce overfitting, the number of peaks was restricted to 2255 by rising thresholds, and using peaks appearing in at least in 95% of exhalations.

c) MS spirometry profile is species dependent, and correlates well with blood-air partition coefficient. To account for this, CO2 signal was used to differentiate lung fractions.

d) Consecutive exhalations show increasing or decreasing signals and reach a steady state in about 6 exhalations. Signal trends are repeatable, and they are species dependent, also correlating well with blood-air partition coefficient. We hypothesize that this is caused by the change in the breathing pattern.

e) Once the steady state is reached, PCA analysis of 2,255 features shows that inter-subject variations are more significant than intra-subject and instrument time-drifts.

Conclusions

With several thousand peaks detected, identification of reliable breath biomarkers with SESI-HRMS is limited by confounding factors. A series of procedures aiming at mitigating the effect of confounding factors have been proposed and tested: (i) eliminate sample handling, (ii) guide the exhalation maneuver, (iii) separate alveolar fraction, (iv) restrict the number of peaks studied, (v) reach steady state after changing breathing pattern. Implementing these procedures we found that the variability is dominated by subject to subject variability. This set up is now being used in a multi-center study in Switzerland and China.
## THE TURING EXHIBITION SPACE

<table>
<thead>
<tr>
<th>Start</th>
<th>Finish</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>09:45</td>
<td>Utilizing the US-EPA CompTox Chemicals Dashboard to deliver public access to a Human Volatilome subset of data</td>
<td>Joachim Pleil</td>
</tr>
<tr>
<td>09:50</td>
<td>10:20</td>
<td>Standardization procedure for exhaled breath analysis using secondary electrospray ionization mass spectrometry</td>
<td>Bettina Streckenbach</td>
</tr>
<tr>
<td>10:20</td>
<td>10:40</td>
<td>Saliva screening for rapid organophosphate poisoning assessment: A case study with preliminary observations and findings</td>
<td>Andria Hadjitheki</td>
</tr>
<tr>
<td>10:40</td>
<td>11:00</td>
<td>Investigating chorioamnionitis in animal model by exhaled breath analysis</td>
<td>Agnieszka Smolinska</td>
</tr>
<tr>
<td>11:15</td>
<td>11:45</td>
<td>Baseline breath volatiles of healthy Non-Human Primates</td>
<td>Jannatu Azmir</td>
</tr>
<tr>
<td>11:45</td>
<td>12:05</td>
<td>Skin volatile profiling using gas chromatography-mass spectrometry as a means to track health status</td>
<td>Aoife Morrin</td>
</tr>
<tr>
<td>13:25</td>
<td>14:10</td>
<td>Detecting Opioid Metabolites in Exhaled Breath Condensate (EBC)</td>
<td>Cristina Davis</td>
</tr>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Human Exhaled Breath Condensate and Aerosol Collection in the Clinical Setting - Techniques, Concerns, and Considerations</td>
<td>Michael Davis</td>
</tr>
<tr>
<td>14:35</td>
<td>14:55</td>
<td>EBC analysis in a pre-clinical study: determining optimal analysis strategy</td>
<td>Agne Krilaviciute</td>
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<tr>
<td>15:30</td>
<td>15:55</td>
<td>Study Design and Clinical Applications of Breath Analysis</td>
<td>Stephen Fowler</td>
</tr>
<tr>
<td>15:55</td>
<td>16:15</td>
<td>Developing Metrology Capabilities to Underpin Breath Analysis</td>
<td>Sergi Moreno</td>
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<td>16:15</td>
<td>16:35</td>
<td>Diet and other factors affecting the variability of breath composition</td>
<td>Fabio Di Francesco</td>
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Breath related research has grown beyond targeted analysis of small groups of gas-phase compounds by adding non-targeted (discovery) strategies with increasingly sophisticated instrumentation. Many hundreds (maybe thousands) of new features in breath, exhaled breath condensate (EBC), exhaled breath aerosols (EBA), and other breath-related in vitro samples including tissue and cell cultures are being identified. To help track and organize new compounds, we have recently implemented a “Human Volatilome” list as part of the U.S. Environmental Protection Agency (EPA) CompTox Chemicals Dashboard. Herein, compounds are assembled into the underlying chemical registration system and made available via the publicly accessible dashboard web interface. This provides access to the chemical structures, CAS Registry Numbers, systematic and alternate names, masses and formulae, experimental and predicted physicochemical properties, available toxicity data, details regarding presence in consumer products, and integration with multiple third party sites including Pubmed Central. The subset of data (~800 at present) is openly available at: https://comptox.epa.gov/dashboard/chemical_lists/VOLATILOME and currently contains all of the compounds listed in the JBR article, deLacy Costello, et al. 2014; new compounds will be added in periodic updates. The dashboard provides access to data for ~875,000 chemicals in total and encompasses a much larger data set than that examined in breath research. This presentation demonstrates the utility of the database and solicits input from the breath community.


Bettina Streckenbach
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(2) Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Switzerland.
(3) Division of Respiratory Medicine and Childhood Research Center, University Children’s Hospital Zurich, Switzerland,
(4) Department of Pulmonology, University Hospital Zurich; Zurich, Switzerland

Background
Secondary electrospray ionization mass spectrometry (SESI-MS) enables real-time screening of a broad range of breath derived compounds, offering high sensitivity together with high mass resolution and accuracy. Accordingly, this non-invasive technology has already been applied in different clinical trials in the discovery of disease-specific biomarkers in exhaled breath. While its applicability seems promising, as potential sets of biomarkers were found e.g. for obstructive sleep apnoea, standardization procedures for this fairly young methodology are currently missing. To achieve comparability of data generated at different time points, on different mass spectrometers and different laboratories, methodological standardization is necessary.

Methods/ Results
We are implementing a stable delivery and dilution system for a gaseous standard mixture tailored for SESI-MS. This reference gas mixture must incorporate compounds of low volatility within various chemical classes, consist of fairly high molecular weight (50 to 300 Da) and at a low molar fraction (ppbv or lower) to optimally cover the method´s range of detectability. Compounds that are detected in breath by SESI-MS are of particular interest. We propose to introduce this mixture from a gas cylinder into a delivery module which enables us to dilute and humidify the mixture to optimally simulate exhaled breath. Both, the delivery system together with the gas mixture composition are evaluated for their suitability. The set-up will finally be validated on different high-resolution mass spectrometers (TripleTOF® 5600+ and Orbitrap Q Exactive) as well as at three different sites in Zurich (ETH Zurich, University Children's Hospital and University Hospital Zurich).

Conclusions
Such a reference gas mixture allows for data comparability and precise monitoring of instrumental stability, both of which are urgently needed for long-term clinical trials. Identifying technical variations in specific, will be fundamental for robust data analysis and, in turn, improve the reliable identification of biomarkers in breath.
31. Saliva Screening For Rapid Organophosphate Poisoning Screening: A Case Study With Preliminary Observations And Findings

Andria Hadjithekli

10:20

Hadjithekli A(1), Dhanarisi J ((2) Eddleston M(3), Salman D(1), Ruszkiewicz DM (1), Joyce A ((1),
Manek A ((1), Palangasinghe C((2), Pearson M ((3), Gawarammana I (2,4) and Thomas CLP ((1)
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Loughborough, UK
(2) South Asian Clinical Toxicology Research Collaboration, University of Peradeniya, Peradeniya,
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of Peradeniya

Background

TOXI-triage, (Horizon 2020 with 18 teams from 8 different countries) seeks to develop and
test non-invasive volatile organic compounds (VOC) screening techniques for the rapid
diagnosis of poisoning or irradiation associated with chemical, biological, radiological and
nuclear (CBRN) incidents. An important element of the research is the realisation of multi-
use applications. To this end, one of the project's 15 clinical studies is developing and
accessing saliva screening as a toxicity diagnostic, and prospecting for markers of
phosphorus (OP) poisoning. Agricultural pesticides are a major contributor of self-harm
deaths in rural-Asia. Data supported by the National Crime Records Bureau and World
Health Organisation (WHO) suggest 35,500 (38.5% of Global Burden) are due to
intentional pesticide poisoning in South East Asia Region. The specialised toxicology unit
in Teaching Hospital Peradeniya (THP) in Sri Lanka is a centre of excellence in the
treatment of self-poisoning cases, and this research was conducted with participation from
the patients admitted to THP due to acute OP insecticide self-poisoning.

Methods

The study was conducted in accordance with the ethical principles of Good Clinical Practice
and the Declaration of Helsinki and the Research Project No. 2017/EC/64 was approved
by the Institutional Ethical Review Committee, Faculty of Medicine at University of
Peradeniya. 43 men and 5 women in the age range 16 to 78 yr were recruited after clinical
admission for OP poisoning (n= 39) or unknown pesticide poisoning (n=9). 24 control
samples (n=6) were also obtained as part of a peppermint evaluation study. A saliva VOC
sample was collected by clinical staff on admission to the research and then at 1hr, 3hr
and 6hr.¹ The samples were stored in air-tight thermal desorption tubes at 4 °C and
shipped to Loughborough University for analysis by thermal desorption gas
chromatography mass spectrometry (Rtx-5MS Cap. Column 60m; 0.25 mm, ID; 0.25 μm,
40°C; 5°C min 1; 300°C hold 8 min). Quality assurance and quality control measures
enabled sample integrity to be monitored throughout the workflow, with a QC/ retention-
index standard mixture run every 3 samples. Data were deconvolved with Spectral Works
AnalyzerPro™ and a procedure was developed to identify the optimum levels for thresholds
for area, height, width and signal to noise ratio; this process accounted for sample
dependent variation in the data. The deconvolved data were evaluated for the presence of
pesticide associated VOC.

Results
Saliva profiles appear to be unique to each participant. Approximately 350 deconvolved features were isolated from each sample and these were evaluated for the presence of exogenous and endogenous features. Classes of compounds recovered include acids, aldehydes, ketones and hydrocarbons. The intensities of features vary from 50 kCounts up to 106 MCounts with siloxanes being the most intense feature. Artefacts of siloxanes from the sample cartridge were exploited for alignment and data registration during data processing. At the time of writing, 9 ubiquitous compounds have been identified in saliva VOC profiles and these enable secondary alignment of the data for the creation of a saliva VOC matrix. Of substantial interest is the presence of halogenated compounds, which are being assessed for their possible utility as OP exposure markers.

Conclusions

At this stage caution needs to be exercised in assigning specific identities to features of interest, and care taken to avoid claims that subsequently are found to be based on false discovery. However what is of interest are the presence of components that may attributable to OP formulations, and in some instances the active poison. Evidence will also be presented for the presence of markers of metabolic derangement and recovery from OP poisoning episodes. Further the possibility of a systemic toxicity marker will be explored.

¹ H.J.Martin et al., Analyst, 2012, 137, 3627, DOI: 10.1039/c2an35432b
Investigating chorioamnionitis in animal model by exhaled breath analysis

Agnieszka Smolinska

10:40

Smolinska A (1), Boots A (1), Dallinga J (1), Ophelders D (2), Wolfs T (2), van Schooten FJ (1),

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(2) Laboratory of Pediatrics, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands

Background

Breathomics can be defined as the metabolomics study of exhaled air. It is a strongly emerging metabolomics research field that mainly focuses on health-related Volatile Organic Compounds (VOCs). Since the composition of these compounds varies depending on health status, breathomics holds great promise as non-invasive diagnostic tool. Breathomics studies usually aim to find the patterns of VOCs associated with abnormal (for instance inflammatory) metabolic processes occurring e.g. in the human body. Consequently, methods for measuring VOCs in exhaled air for diagnosis and monitoring health status gained increased attention over the last years and have enormously developed. Breathomics has been demonstrated as useful research field to monitor and diagnose diseases in various clinical as well as animal studies.

Methods

In the current study exhaled breath was used as a potential diagnostics tool for chorioamnionitis, intra-amniotic septicity due to bacteria infections. An animal model using sheep was used to investigate whether the inflammation of the fetal membranes can be detected using exhale breath analysis. In the discovery set, four pregnant sheep were used to induce chorioamnionitis by injecting ueroplasma species into the amniotic cavity. Exhaled breath samples were collected at baseline and at daily basis for the period of 7 days. The exhaled breath was first collected to Tedlar bag and immediately transferred onto stainless steel desorption tubes (1TD/Carbopack X) and analyzed by GC-tof-MS. After careful data pre-processing, the data were subsequently analysed by three-based techniques to find the volatile metabolites in exhaled breath that were specific to intra-amniotic infection. In the final step the discriminatory volatile metabolites were validated in the independent set of four sheep.

Results

The statistical analysis revealed distinct differences in the content of exhaled breath five days after injecting ueroplasma species into the amniotic cavity. The set of 15 VOCs was found to have the highest contribution in discriminating baseline measurements and those obtained five days after infection. The obtained results were further validated in the independent set of four sheep. The validation of the set of the discriminatory VOCs led to sensitivity of 83% and specificity of 71%. The set of discriminatory compounds were putatively identified as linear alkenes, alcohol and acids.

Conclusions

To the best of our knowledge, this is the first study where exhaled breath is used in sheep to investigate the effect of chorioamnionitis. The results demonstrated that exhaled breath might be successfully used to diagnose the inflammation of the fetal membranes.
Jannatu Azmir

11:15

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(2) Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

(3) Department of Pediatrics, Division of Infectious Disease, Children’s Hospital of UPMC, Pittsburgh, Pennsylvania.

Background

Non-human primates (NHPs) are so similar to humans that they are used to study over 70 different human infections and many other diseases. And, they are used pre-clinically to determine the efficacy of drugs and vaccines. But, NHPs are expensive to use and importantly, are sentient beings, so their use should be limited as much as possible. Here, we propose that breath monitoring could be used to limit costs and limit use of NHPs. We propose that there is a healthy breath volatile organic compound profile and that this baseline breath profile can be used in basic and pre-clinical studies of NHPs.

Methods

We conducted two studies. The first was to develop a standard operating procedure for the collection of breath in macaques (rhesus and cynomolgus). The second was to develop baseline breath profile of healthy NHPs. A total of 16 healthy NHPs were evaluated in this study. Macaque breath from each animal was collected at two unique times (n=16×2=32). And, these same animals were given a low dose of (3-13 CFU) of M. tuberculosis Erdman strain. Room air samples were also collected during sampling. Breath was collected in a 5-liter Tedlar bag and concentrated through a 0.22-µm filter (for the removal of potential pathogens), and onto the thermal desorption (TDT) tube.

Breath and air samples were analyzed by a comprehensive two-dimensional gas chromatography with time of flight mass spectrometry (GCxGC-TOFMS). Some statistical analysis were carried out on the obtained VOC features to find the core (that are presented in all macaques sample) and pan volatilomes as the baseline breath volatiles of healthy NHP.

Results

We developed the breath collection method from an anesthetized animal into a Tedlar bag and subsequent concentration onto a thermal desorption tube for storage. Breath was analyzed and found that breath had a different composition to room air, though many features overlapped. The following chemical compound classes; alcohols, aldehydes, aromatics, carboxylic acids, esters, hydrocarbons, ketones, and halogen, nitrogen and sulfur-containing molecules were detected in the breath. We also identified the core (that are found in all macaques samples) and pan-volatilomes as the baseline breath VOCs for NHPs.

Conclusions

In this study, exhaled breath was successfully collected from anesthetized macaques. The baseline breath VOCs profile of healthy NHPs was also identified with 136 core volatilomes.
Skin volatile profiling using gas chromatography-mass spectrometry as a means to track health status

Aoife Morrin

11:45

Aoife Morrin(1)

(1) Insight Centre for Data Analytics, School of Chemical Sciences, Dublin City University, Dublin 9

Background

Systemic disease as well as localized skin disease modify the molecular composition of human skin. Changes in skin chemistry occur in diseases such as cancer, psoriasis, eczema, diabetes and inflammatory bowel disease. We are interested in understanding how the skin volatile profile is affected in the presence of disease and have been studying the human skin volatile profile with a view to identifying patterns that would permit diagnosis of disease or tracking of flare-ups in disease.

Methods

Our research typically employs a glass-based headspace-solid phase microextraction (HS-SPME) approach to collect skin volatile samples, and GC-MS is used for analysis. The sampling site is the inner forearm unless otherwise specified.

Results

To date, we have conducted studies in healthy participants to characterise skin volatiles and to understand their variability across a population. Volatile compounds detected are discussed in terms of origin, i.e., glandular secretions, bacterial, exogeneous, etc. We are also interested in studying the change in skin volatile profile when the skin barrier becomes compromised. This is relevant to certain disease states such as atopic dermatitis (AD). We show that skin volatiles profiles are impacted when the skin barrier is disrupted.

Conclusions

An understanding of the baseline skin volatile profile and the factors that influence the profile has been developed. Despite being highly variable in nature, significant differences in skin volatile profiles across distinct sub-groups in healthy populations has been demonstrated. Early clinical data is also being generated which will be discussed to support the hypothesis that skin volatile profiling can add value in aspects of personalised health status tracking.
Volatile compounds present in human breast milk are thought to play an important role in maternal-infant bonding, however, the compound(s) responsible for this apparent “scent signal” have not yet been elucidated. Untargeted metabolomic profiling using highly sensitive analytical instrumentation could aid in the identification of these compounds, with potential implications for infant health and nutritional status.

Methods

Human milk was collected from 43 mothers at their six-week postpartum appointments, and the volatile molecules present in the headspace of these milk samples were concentrated using HS-SPME and analyzed via comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry GC×GC-TOFMS.

Results

506 unique volatile compounds were detected in the headspace of human milk samples. Of these, 188 (37 %) were common to all mothers, while the remaining 318 were detected in only a subset of samples. One hundred and forty-eight (29 %) compounds could be assigned names based on mass spectral matching, of which 97 have not previously been detected in human milk.

Conclusions

Human milk contains a greater number and wider variety of chemical compounds than previously reported using other analytical techniques. While the precise identities of the volatile compounds responsible for maternal-infant signaling remain undetermined, the novel compounds reported in this study represent potential candidates.
Cristina Davis

13:30

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Background

Exhaled breath condensate (EBC) collection provides a promising matrix for bioanalysis of endogenous biomarkers of health and also for exogenous compounds like drugs. There is little information regarding drugs and their metabolites contained in breath, as well as their pharmacokinetics. In this present work, we use a simple and non-invasive technique to collect EBC from chronic pain patients using different analgesic opioid drugs to manage pain. Six patients received continuous infusion of morphine and hydromorphone intravenously (IV), together with other analgesic drugs (IV and orally).

Methods

Repeated sampling of serum and EBC was done at two time points separated by 90 min. The EBC was collected using a glass tube surrounded by dry ice, and an ethanol solvent wash of the glass was performed after EBC extraction to retrieve the apolar compounds stuck to the glass surface. All samples were analyzed with liquid chromatography coupled to mass spectrometry (LC-MS/MS) to identify possible metabolites present in the sample, and to quantify the drugs being used.

Results

Several metabolites, such as normorphine (norM), norhydromorphone (norHM) and dihydromorphone (diHM) were detected in both fractions, while hydromorphone 3-
THE TURING EXHIBITION SPACE

gluconide (HM 3G) was only detected in the solvent rinse fraction. Results were correlated to explain the pharmacokinetics of the main drugs administered.

Conclusions

This pilot study presented promising correlations between drug concentrations in blood and breath at different time points for norM, norHM and HM 3G.
Human Exhaled Breath Condensate and Aerosol Collection in the Clinical Setting - Techniques, Concerns, and Considerations

Michael Davis

14:15

Davis, MD

(1) Wells Center for Pediatric Research at the Indiana University School of Medicine

Human exhaled breath condensate (EBC) and aerosol (EBA) are promising matrices of respiratory and systemic biomarkers that are particularly convenient for researchers due to the non-invasive nature of their collection.

When collecting samples in the clinical setting, care must be taken for quality assurance and, more importantly, safety to the research team and subject. This presentation will provide an overview of collection techniques for EBC and EBA collection specific to the clinical setting and also of safety and quality concerns for this environment and population.
EBC analysis in a pre-clinical study: determining optimal analysis strategy

Agne Krilaviciute
14:35
Krilaviciute A (1), Mueller T (2), Hessling B (3), Schrotz-King P (1), Brenner H (1, 2, 5)
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(2) Division of Proteomics of Stem Cells and Cancer,
(3) Genomics and Proteomics Core Facility, (4) Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany
(5) German Cancer Consortium (DKTK), Heidelberg, Germany

Background
We tested a newly developed exhaled breath condensate (EBC) collection device in a pre-clinical study for lung cancer early detection with EBC analysis using 100 clinically diagnosed lung cancer and 100 age- and sex-matched cancer-free controls. Here, we report our learnings from additional 200 samples collected for device calibration using the same study protocol approach.

Methods
On average, 50 liters of exhaled breath yielded 530 µl of EBC per individual. Collected EBC was divided into 4 aliquots (200 µl, 100 µl, 100 µl and the remaining volume), where 2 first aliquots had protein inhibitors added before freezing them at -80C. Samples were analyzed using liquid chromatography-mass spectrometry (LCMS) in a total of 8 analyses, and in this abstract we focus on selected 3-step approach of our calibration phase: in Step 1, we analyzed various sample sizes (with and without protein inhibitors) to determine the minimum required volume for optimal protein yield for the LCMS analysis with single-pot solid-phase-enhanced sample preparation with the paramagnetic beads (SP3) protocol: 100 µl (n=10) and 500 µl (n=12), as well as 200 µl, 300 µl, 400 µl (n=2 each). In Step 2, we analyzed another 13 samples with the protein inhibitors and 7 samples without protein inhibitors (volume, 300 µl) to confirm initial findings from the step 1. In Step 3 we analyzed n=20 samples (with and without protein inhibitors, volumes 300 µl and 500 µl that makes n=5 samples per group) using in-gel digestion for sample pre-processing for LCMS analysis.

Results
In Step 1, substantially less proteins were detected in 100 µl (<10 per sample) as compared to 500 µl (on average 60 proteins per samples), while at least 300 µl appeared to be necessary for non-inferior protein detection. However, samples with protein inhibitors appeared to yield fewer proteins as compared to same-volume samples without protein inhibitors. In step 2, analysis of 20 additional samples (volume, 300 µl) indicated that protein inhibitors interfere with the SP3-LCMS analysis, with only 3 out of 13 samples yielding more than 30 proteins per sample compared to 4 out of 7 samples without protein inhibitors. In Step 3, we found no difference in the number of detected proteins per samples with or without protein inhibitors when using in-gel digestion for sample preparation: on average, 39 and 34 proteins, respectively, were detected in 300 µl, as compared to 76 and 79 proteins in 500 µl.

Conclusions
THE TURING EXHIBITION SPACE

1. The sample work-up factors may have substantial influence on the protein yield per sample in LCMS analysis.

2. It is crucial to pre-test new sample collection devices and sampling protocols, as well as to report the routine procedures applied in clinical studies using EBC samples.

3. From the learnings in our study, we will go ahead in the clinical phase with the in-gel digestion and use the total collected sample volume for future comparative analysis of 100 lung cancer patients and 100 cancer-free controls.
Stephen Fowler
15:30
Fowler SJ(1)
(1) Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, and
(2) Manchester Academic Health Science Centre and
(3) NIHR Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, Manchester, UK

Over the last two decades there has been a rapid rise in investigations reporting potential clinical applications for breath volatiles, such that we are now at the stage where systematic reviews are not only feasible but being published in major journals. Careful review of these offers the opportunity to consolidate both (rare) common findings as well as (frequent) differences between studies. We should use these to inform future directions in breath analytical research, always with an eye on future clinical effectiveness studies, and ultimately implementation. We will discuss issues and potential solutions for the clinical breath platform, in particular around diagnostics, phenotyping, treatment monitoring and biological validation. Study quality assessment in systematic reviews will also be discussed, as this could help inform future study design if considered at an early stage.
The studies in the area of Breath Analysis have grown considerably in the last years due to the development of new analytical instruments such as Proton Reaction Mass Spectrometry (PTR-MS), Selective Ion Flow Mass Spectrometry (SIFT-MS) and Secondary Electrospray Ionisation Spectrometry (SESI-MS). However the lack of standardisation is a barrier to the adoption of breath tests in clinical practice.

Widespread diversity in sampling and measurement methods and the lack of reference materials contribute to the poor comparability of clinical trial results, which inhibits the verifiable identification of VOC biomarkers. MS libraries can lead to incorrect identification of peaks even at FM > 800. Recent recognition that variation in amount fraction of biomarkers is more important than qualitative detection of unique markers also drives the requirement for more accurate quantitation.

The National Physical Laboratory (NPL) maintains Primary Reference Materials (PRMs) that sit at the top of the traceability chain with the smallest uncertainties. It is only by comparison of these primary realisations of the mole with other National Metrology Institutes and Designated Institutes around the world, using the measurement redundancy that this provides, that the full uncertainty of the realisation can be achieved.

We present developments at NPL in reference materials for Breath Analysis: A PRM for selected compounds of the Peppermint Project and a novel PRM for PTR-MS calibration containing 20 different VOCs. These developments will make a significant impact on the breath analysis community ensuring comparability and facilitate longitudinal studies.
THE TURING EXHIBITION SPACE

41. Diet and other factors affecting the variability of breath composition

Fabio Di Francesco

16:15

Lomonaco T (1), Fusi J (2), Bellagambi FG (3), Ghimenti S (1), Biagini D (2), Franzoni F (2), Fuoco R (1), Di Francesco F (1)

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(2) Department of Clinical and Experimental Medicine; University of Pisa, Pisa, Italy;
(3) Institut des Sciences Analytiques; Université Claude Bernard Lyon 1, Lyon, France

Background

Exhaled breath contains information concerning exposure to xenobiotics, food and beverage consumption, normal and abnormal physiology and presence of bacteria. Such richness of information results in a large intra- and inter-individual variability of breath composition, which is further increased by sampling conditions, circadian rhythms and diet. Variability hinders the identification of specific biomarkers or “breathprints” and makes it complex to tell pathological from healthy subjects apart.

Methods

Breath composition was analysed in 20 healthy subjects following an omnivorous (n= 10) or a vegan (n= 10) diet under different conditions. Mixed breath was sampled by asking each subject to fill a Nalophan bag, then 100 mL of sample were loaded into a sorbent tube (250 mg of Tenax GR ,70/80 mesh) before being analysed by thermal desorption coupled to gas chromatography / mass spectrometry.

Results

Respiratory rate and ventilation modified breath composition, where the largest variations were observed with isoprene and acetone. Vegans showed lower values of VOCs related to oxidative stress compared to omnivorous subjects, but differences were not very large. Individual behaviours concerning smoke or consumption of specific foods were also mirrored in breath.

Conclusions

Even if some information is available in literature, more and more experiments are showing that variability of breath composition in nominally healthy subjects is larger than previously expected. The breath community should put more emphasis in understanding the role and the relative importance of the sources of such variability as part of the work in standardizing breath measurements.
<table>
<thead>
<tr>
<th>Start</th>
<th>Finish</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:50</td>
<td>10:20</td>
<td>Real-time breath analysis during exhaustive exercise on a cycle ergometer</td>
<td>Giovanni Pugliese</td>
</tr>
<tr>
<td>10:20</td>
<td>10:40</td>
<td>Circadian rhythm of exhaled biomarkers in health and asthma</td>
<td>Max Wilkinson</td>
</tr>
<tr>
<td>10:40</td>
<td>11:00</td>
<td>Determination of VOCs in breath samples and their potential to assess diagnosis of respiratory diseases</td>
<td>Ileana Andreea Ratiu</td>
</tr>
<tr>
<td>11:15</td>
<td>11:45</td>
<td>EVOC Probes For The Assessment Of Metabolic Pathways: Using Breath Limonene To Assess The Impact Of Liver Disease</td>
<td>Rob Smith</td>
</tr>
<tr>
<td>11:45</td>
<td>12:05</td>
<td>Stable isotope or unlabelled-probe breath tests vs endogenous VOC’s breath tests - a review</td>
<td>Anil Modak</td>
</tr>
<tr>
<td>12:05</td>
<td>12:25</td>
<td>Fighting Anti-Microbial Resistance With Breath Analysis</td>
<td>Emma Brodrick</td>
</tr>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Modelling electronic nose sensor deflections by matching Gas</td>
<td>Paul Brinkman</td>
</tr>
<tr>
<td>14:15</td>
<td>14:35</td>
<td>Chromatography-Mass Spectrometry analysed exhaled breast samples</td>
<td>Paul Brinkman</td>
</tr>
<tr>
<td>14:35</td>
<td>14:55</td>
<td>Prospective Early Detection of Lung Cancer in COPD Patients by Electronic Nose Analysis of Exhaled Breath</td>
<td>Rianne de Vries</td>
</tr>
<tr>
<td>14:25</td>
<td>15:55</td>
<td>Role of Breath Based Volatile Organic Compounds in Detection of Gastroesophageal Disorders</td>
<td>Ravi Vissapragada</td>
</tr>
<tr>
<td>16:15</td>
<td>16:35</td>
<td>Multimodal breath-based asthma phenotyping using GCxGC-HRTOFMS and SIFT-MS approaches</td>
<td>Pierre-Hugues Stefanuto</td>
</tr>
</tbody>
</table>
Giovanni Pugliese

09:50

Pugliese G(1), Trefz P (1), Sukul P(1), Weippert M(2), Bruhn S(2), Miekish W(1), Schubert JK (1)

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(2) Institute of Sport Science, University of Rostock, Ulmenstrasse 69, 18057 Rostock, Germany

Background

Exhaustive exercise causes changes in energy consumption, fuel utilization and metabolic conditions (e.g. aerobic and anaerobic metabolism) in the body. The aim of the present study was to monitor these changes non-invasively and in real-time via exhaled volatile organic compound (VOC) concentration profiles.

Methods

Fifteen healthy human subjects performed an incremental cycle ergometer ramp test. After a standardized warm-up of 5 min at 50 W, all participants performed a ramp protocol with increments of 25 W/min until exhaustion immediately followed by 5 minutes of cooldown at 50 W. Maximal effort was reached when the volunteer was unable to maintain a pedal cadence of 60 rpm.

Breath VOC concentrations were monitored in real-time by means of PTR-ToF-MS 1000. Breath was sampled continuously in side-stream mode by means of a heated 6 m silcosteel transfer line while the participant was breathing through a sterile facemask. The mask was adapted for continuous PTR sampling via a Teflon piece with a side stream Luer connection where the PTR transfer line was connected. Simultaneously, respiratory and hemodynamic parameters were monitored noninvasively and in real-time by means of metamax device and ClearSight System EV1000, respectively. Heart rate (HR) measurement was done via a chest strap. Capillary blood samples were taken at each increment from the earlobe and blood lactate concentration was determined photometrically.

Results

Isoprene concentration increased by 9% at begin of the exercise and then steadily decreased down to 25% of the base levels at the end of the workload. Acetone concentration reached a maximum at about 90% of the total workload, and then decreased until the end of exercise. Acetonitrile, acrolein , methanethiol and butanal levels showed a significant increase at the begin of the exercise and then a steady decrease down to 38%, 58%, 70% and 72% of the baseline levels at the end of the workload. Blood lactate and spiro-ergometric parameters like oxygen consumption (VO2), carbon dioxide production (VCO2), minute ventilation (VE), tidal volume (VT) and heart rate (HR) steadily increased from the beginning of exercise until the end of the test.

Isoprene, butanal and acrolein showed significant correlations with VO2. The individual anaerobic thresholds determined by means of acetone breath concentration profiles were in agreement with those determined by means of blood lactate curves.

Conclusions
Breath VOC concentrations as well as spiro-ergometric parameters showed significant changes during exhaustive exercise. Breath markers may provide a non-invasive insight into metabolic and physiological processes in the whole body.
Max Wilkinson

10:20

Wilkinson M(1), Maidstone R(2,3), Loudon A(4), Blaikley J(1,5), White IR(1,6), Singh D(1,7), Ray DW(3), Goodacre R(8,9), Fowler SJ(1,5), Durrington HJ(1)

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(7) Medicines Evaluation Unit (MEU), Langley Building, Manchester University Hospitals NHS Foundation Trust, South-moor Road, Manchester, UK

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(9) Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK

Background

Circadian rhythms control many biological processes in the body in both health and disease. Greater understanding of diurnal variability in disease related biomarkers is crucial for their application in clinical practice and biomarkers of circadian rhythm are required to facilitate further research into disturbed chronicity. To determine if fractional exhaled nitric oxide and breath volatile biomarkers vary rhythmically during the day in healthy and asthmatic individuals.

Methods

Ten individuals with moderate, atopic asthma (on regular inhaled corticosteroids) and 10 healthy volunteers (all non-smokers) completed an overnight visit where their exhaled breath volatiles and forced exhaled nitric oxide levels were collected every 6 hours. Breath volatiles were analysed using gas chromatography mass spectrometry, after trapping these volatiles on sorbent materials for thermal desorption.

Results

Nine breath volatiles (including acetone and isoprene) exhibit diurnal variation across all individuals. Furthermore the circadian pattern of several VOCs is altered in individuals with asthma and fractional exhaled nitric oxide is rhythmic in asthma but not in healthy controls.

Conclusions

Markers of circadian rhythm can be identified in breath and may offer insight into circadian profiling to help treat disease. Additionally this work suggests that time of day must be
controlled when designing future biomarker discovery studies. Further work is required with larger cohorts to validate and extend these findings.
44. Determination of VOCs in breath samples and their potential to assess diagnosis of respiratory diseases

Ileana Andreea Ratiu

10:40

Ratiu IA (1,2), Monedeiro F (1), Milanowski M (1), Ligor T (1), Buszewski B (1).

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(2) Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj Napoca, Romania.

Background

Metabolic processes occurring naturally in our organisms lead inherently to the formation of many volatile organic compounds (VOCs). In case of any pathology, differentiated processes unwind in the cells, generating a new, atypical, set of metabolites. Assessment and characterization of biochemical changes in a living organism, reflected in the volatiles released, represent a promising approach that can lead to fast and non-invasive diagnosis tools for various diseases. Breath is a complex biological matrix that closely corresponds to the respiratory system and consequently to blood, but presenting a less complex composition and far simpler sample collection compared with others. Breath tests are being, most probably, the least invasive and therefore the most easily accepted by the patients. Exhaled breath samples consist normally of three fractions: gaseous breath, volatile breath, and breath condensate. Just about 1% of the total volume of a breath sample is represented by volatile fraction, which contains hundreds of detectable and quantifiable compounds. This work aimed to quantify 29 target VOCs previously reported as biomarkers of pulmonary diseases and, additionally, to investigate the global VOCs profiles in breath samples, in order to check for variations in compounds distribution along the studied groups of subjects.

Methods

Breath samples were collected in Tedlar bags, both from healthy individuals and from volunteers with diagnosed respiratory diseases, recruited in local hospital dependencies. Dynamic solid phase microextraction (SPME) and needle trap device (NTD) were employed for VOCs sampling. The analyses were carried out by an Agilent 5975 Inert XL MSD gas chromatograph coupled to mass spectrometer GC-MS, equipped with a DB-624 (Agilent) column (60m x 0.32mm x 1.8µm). The injector temperature was 220°C, while ion source and transfer line were set at 220°C. Oven program is described by five different gradients of temperature, resulting in a total run time of 41.33 min. The full scan range was 20 m/z to 300 m/z.

Results

Calibration curves were built using six levels of concentrations, each one run in triplicate. Limits of quantification ranged from 0.55 to 4.44 ppbv for Dynamic SPME and from 0.59 to 18.08 ppbv for NTD. Relative standard deviation (RSD) did not exceed 10%, in both methods. The obtained data were assessed using statistical approaches (Mann-Whitney U test, principal components analysis and hierarchical cluster) and results revealed that at least 17 targets presented were differentially distributed in samples from healthy and diseased individuals. After profiles' investigations, over 80 compounds could be detected by the applied methodologies; some of them presented discriminating features according with the investigated group.

Conclusions
The developed methodology proved to be suitable for the determination of target compounds of interest in breath samples. The use of different techniques for sample extraction resulted in obtaining of a comprehensive database of VOCs. The selected analytes provided differentiation by their own increased response in the positive samples.

Acknowledgments: This work was financed by The National Centre for Research and Development (Warsaw, Poland) in frame of Polish-Turkish bilateral project "A comparative study of volatile organic compound biomarkers in breath and urine samples collected from Polish and Turkish communities for monitoring of various respiratory diseases" (POLTUR2/4/2018).
**Background**

Recent studies have demonstrated the concentration of limonene on breath is significantly higher in pre liver transplant patients as compared to healthy individuals [1-3]. Interestingly, following liver transplantation a gradual reduction in limonene breath levels are observed [2]. This suggests a link to improved liver function and more specifically an increase in CYP2C9 and CYP2C19 enzymes in the liver, enzymes which have been demonstrated to metabolise limonene. The objective of this study is to assess whether exhaled limonene levels change in patients with various stages of liver cirrhosis and hepatocellular cancer (HCC) as compared to controls.

**Methods**

Patients with confirmed liver cirrhosis, patients with confirmed HCC and healthy individuals will be recruited to this trial. Patients with acute hepatic failure will be excluded. Breath samples will be captured on Tenax sorbent tubes using the ReCIVA Breath Sampler. Samples will be analysed by TD-GC-MS using the Breath Biopsy workflow.

Limonene concentrations will be compared between the patient groups and between disease severity classes. It is expected that patients with liver cirrhosis will have increased levels of limonene in their breath. A pre-trial analytical verification was performed on individuals pre and post drinking 1ltr of orange juice collecting different breath volumes to demonstrate method linearity within breath samples. Data will be reported on methods of limonene administration and study progress.

**Conclusions**

This study will investigate associations between exhaled limonene concentrations and various liver pathologies. As such it could provide proof of concept evidence for using eVOC probes for the assessment of individual metabolic pathways. We believe this underpins the importance of understanding the metabolism of VOCs to develop sensitive and specific diagnostic breath tests.
Anil Modak

11:45

Dr Anil Modak

Background

In the modern era since Linus Pauling's VOC microanalysis in 1971, breath tests have been extensively researched both with endogenous VOC's as well as the more specific 13C-probe based breath tests using 13CO2 as a biomarker of physiological changes and genetic diversity in humans.

With the development of sensitive analytical techniques, we can now investigate for unique biomarkers in a person's breath to identify particular medical conditions. Breath analysis has the potential to become a non-invasive diagnostic tool in clinical practice.

Over the last two decades non invasive diagnostic phenotype [13C]-breath tests, as well as tests using endogenous volatile organic compounds (VOCs) in breath have been researched extensively. However, only five breath tests have been approved by the regulatory boards in the US/Europe (FDA/EMA)

1. NO breath test (endogenous VOC) by Aerocrine AB in 2003
2. Heartsbreath test (endogenous VOC's) by Menssana Research, Inc. in 2004
5. LiMAX using Methacetin-13C by Humedics GmbH in 2017-8

Linking VOC’s to specific illnesses has been extremely challenging since oxidative processes in different organs of patients afflicted with various diseases could result in the generation of the exact same VOC’s lowering the ability to pinpoint one of more VOC’s to reliably detect a specific disease. The origins of the VOC’s due to physiological processes in the human body need to be identified for them to be useful as biomarkers of disease.

On the other hand using either stable isotope or unlabeled probes for evaluating various drug metabolizing enzyme deficiencies for personalizing medications have the potential of being the most promising breath tests that can make the transition from research to the clinic. The pros and cons of both breath test paradigms for medical applications will be discussed in great detail.

References

1 Modak AS. An Update on 13C-BreathTests: The Transition to Acceptability into Clinical Practice in Volatile biomarkers: non-invasive diagnosis in physiology and medicine p 245-262. A

Fighting Anti-Microbial Resistance With Breath Analysis

Emma Brodrick
12:05

Brodrick E(1), Covington JA (2), Adams ER (3), Feasey N (3), Skinner JR (2), Radic M (4), Sanders D (5)

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(5)G.A.S. Gesellschaft für analytische Sensorsysteme mbH, Dortmund, Germany

Background
Identification of bacterial respiratory tract infection (RTI) is key to alleviating antimicrobial resistance via improved antibiotic stewardship. The aim of this observational study is to discriminate adult participants with suspected upper or lower RTI with bacterial infection from those without, using breath analysis. Breath samples were tested using a gas chromatography – ion mobility spectrometry (GC-IMS) to measure volatile organic compounds (VOCs) components. Confounding factors, such as age, smoking habits and gender will be investigated to demonstrate the efficacy of results.

Methods
1229 subjects were recruited, all suspected of a RTI from 8 NHS sites a cross the UK. Patients were recruited from both primary (397 subjects) and secondary care (832 subjects) settings. Breath samples were analysed using a commercial GC-IMS (G.A.S. BreathSpec®, Dortmund, Germany).

Results
Initial data analysis indicates that the G.A.S. BreathSpec® GC-IMS was able to separate between diagnostic groups well. Ongoing VOC analysis indicates that certain compounds play a crucial role in distinguishing between diagnostic groups. Analysis of possible confounding factors indicate that gender, age and smoking habits have insignificant influence on breath content.

Conclusions
This observational study confirms the utility of exhaled breath analysis to distinguish between bacterial RTI and those without. This potential diagnostic power would reduce antibiotic prescribing by over 30% in a primary care setting alone. Therefore, the G.A.S. BreathSpec® GC-IMS instrument offers great potential as a non-invasive, high-throughput, diagnostic tool for RTI in a clinical setting while reducing antimicrobial resistance through improved antibiotic stewardship.
Modelling electronic nose sensor deflections by matching Gas Chromatography-Mass Spectrometry analysed exhaled breath samples

Paul Brinkman

14:15

Brinkman P (1), Sinha A (1), Lammers A (1), Van Bragt JJ. (1), Richards LB. (1), Dagelet YW. (1), Ibrahim MI. (1), Neerinx AH. (1), Bos LD. (1,2), Sterk PJ. (1), Maitland-Van Der Zee AH. (1)

(1) Department of Respiratory Medicine.
(2) Departments of Intensive Care Medicine; Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Background

Analysis of expired volatile organic compounds (VOCs) by cross-reactive gas-sensor driven electronic nose (eNose) technology is a widely suggested physiological measure for non-invasive monitoring and management of chronic airway diseases (Bos et al. JACI’16). While this pattern recognition-based strategy allows probabilistic (clinical) advise, it is incapable of providing details concerning involved metabolites. Analytical methods based on mass spectrometry (MS) allow identification of molecules. Modelling eNose sensor deflections by matching Gas Chromatography-Mass Spectrometry (GC-MS) measurements could help to ascertain which groups of VOCs induce a sensor deflection. The objective of this study was to determine the association between eNose sensor deflections and exhaled VOCs measured by GC-MS.

Methods

In this study exhaled breath of asthma patients (n = 22) was collected in a Tedlar bag using a standardized method (Brinkman et al. CEA’17). Paired samples were taken from the bag using sorbent tubes and a gas sampling pump. One tube was analyzed by a composite of four eNose instruments (Brinkman et al. JACI’18) and the second one by GC-MS. Modeling between the two analysis strategies consisted of 1) Partial Least Square Regression (PLSR) analysis [response: eNose, predictor: GC-MS] and 2) computation of a clustered heatmap based on loadings of PLSR analysis to group and reveal sensors and GC-MS features exhibiting highly similar characteristics.

Results

Matching data was available for a total of 158 eNose sensors and 1025 GC-MS features, whereby PLSR analysis resulted in R2’s between 0.25 and 0.72. Heatmapping revealed clear regions of eNose sensors and GC-MS features exhibiting highly similar characteristics.

Conclusions

This explorative analysis revealed distinctive and associated patterns of exhaled VOCs between eNose and GC-MS. This data matching could help to identify which VOCs are responsible for clinically relevant results obtained by eNose and will facilitate valorisation of breathomics into clinical tests.
Prospective Early Detection of Lung Cancer in COPD Patients by Electronic Nose Analysis of Exhaled Breath

Rianne de Vries

14:35

de Vries R* (1,8), Dagelet JWF (1), Farzan N (8), Dijkers E(1), Fabius T(2), de Jongh FHC (2), Jak PMC (3), Haarman EG (3), Kester S(4), Bekkers M (4), Van Den Heuvel MM (5), Baas P (6), in ’t Veen JCCM (7), Maitland-Van Der Zee AH (1), Sterk PJ (1)

(1) Amsterdam University Medical Centers, University of Amsterdam - Amsterdam (Netherlands)
(2) Medisch Spectrum Twente - Enschede (Netherlands),
(3) VU Medical Center - Amsterdam (Netherlands),
(4) Medical Center Den Bosch Oost – Den Bosch (Netherlands),
(5) Radboud University Medical Center - Nijmegen (Netherlands),
(6) The Netherlands Cancer Institute - Amsterdam (Netherlands),
(7) Franciscus Gasthuis en Vlietland- Rotterdam (Netherlands),
(8) Breathomix B.V. – Reeuwijk (Netherlands)

Background

Patients with COPD are at high risk of developing lung cancer [Biswas Curr Opin Pulm Med 2018], but no biomarkers have been reported to identify these patients. Molecular profiling of exhaled breath by electronic nose (eNose) technology may qualify for early detection of lung cancer in COPD patients. The aim of this study was to determine the diagnostic accuracy of exhaled breath analysis by eNose for the prospective prediction of early lung cancer in COPD patients.

Methods

BreathCloud is an ongoing multicentre observational study using diagnostic and monitoring visits in day-to-day clinical care of patients with a standardized diagnosis of asthma, COPD or lung cancer. Exhaled breathprints were collected at inclusion in duplicate by a metal oxide semiconductor eNose positioned at the rear end of a pneumotachograph (SpiroNose) [De Vries ERJ 2018]. All patients with COPD were managed according to standard clinical care and the incidence of clinically diagnosed lung cancer was prospectively monitored for 2 years. Data-analysis involved advanced signal processing, ambient correction and statistics based on principal component analysis (PCA) followed by linear discriminant analysis and receiver operating characteristic (ROC) analysis.

Results

Exhaled breath data of 682 COPD patients were available. 37 COPD patients (5.4%) developed clinically manifest lung cancer within 2 years after inclusion. Principal component 1, 2 and 3 showed a significant difference (p<0.01) at baseline between COPD patients who did and did not subsequently develop lung cancer within 2 years, with a cross-validation value of 89% and a ROC-AUC of 0.90(CI:0.84-0.95). The identification of patients who did develop lung cancer resulted in a sensitivity of 86%, a specificity of 89%, a positive likelihood ratio (LR+) of 7.80 and a negative likelihood ratio (LR-) of 0.15.

Conclusions

Exhaled breath analysis by eNose identified COPD patients in whom lung cancer subsequently manifested within 2 years after inclusion. These results show that eNose
assessment may detect early stages of lung cancer in patients with COPD and may therefore be of value in screening of this risk group.
Ravi Vissapragada

15:30

Vissapragada R(1), Dharmawardana N(1), Watson DIl(1), Yazbeck R(1)

(1) Discipline of Surgery, College of Medicine and Public Health, Flinders University, Adelaide, South Australia

Background

Breath analysis of volatile organic compounds (VOCs) is a promising, non-invasive modality for early detection of oesophageal cancer (OAC) and related conditions. We aimed to characterise previously reported breath VOCs in oesophageal adenocarcinoma and Barrett’s oesophagus (BO) in a South Australian setting.

Methods

Breath samples were collected and analysed prospectively from patients presenting to the Flinders Medical Centre Endoscopy unit using previously standardized. Hydrogen, methane and other VOCs were quantified by Quintron® BreathTracker® and selected-ion flow tube mass spectrometry (SIFT-MS, Syft®) respectively. 13 separate VOCs reported in literature were compared between patient groups and room air. Statistical analysis was performed with Kruskal-Wallis ANOVA (non-parametric data) with Dunn’s posthoc analysis. A p-value < 0.05 was considered statistically significant.

Results

A total of 29 patients were included for this analysis (Benign, n=9; Barrett’s Oesophagus (BO), n=10; Advanced Oesophageal Cancer (OC), n=10). Within the cancer cohort, there was one patient with Stage 2 disease, six with Stage 3 disease, and three with Stage 4 disease. Several previously reported VOC were compared within the groups including: Hexanoic acid, 1-heptene, 2-methyl-phenol, decanal, nonanal, ethyl-phenol, isoprene, acetone, hydrogen sulphide (H2S), heptanal, and butyric acid. Of these compounds, only heptanal and hydrogen sulphide were significantly lower (p<0.05) in the cancer group compared to the benign group. Heptanal had a median value of 19 ppb (IQR 9.7-42.5) compared to 8.475 ppb (IQR 3.5-27.4) benign versus OAC respectively, whereas H2S had a median value of 257 ppb (IQR 71-614) and 24.2 ppb (IQR 7.3-76)

Conclusions

Previous studies have supported the utility of breath VOCs as potential biomarkers of oesophageal cancer. We identified significant differences in H2S and Heptanal in patients with cancer vs. benign pathologies in this small cohort of patients. We are currently expanding recruitment and undertaking comprehensive biomarker discovery analysis to identify novel predictive models for OAC and related conditions.
Non-Invasive Triage Of Symptomatic Fast-Track Colorectal Patients Using Volatile Compounds: A Feasibility Study

Oliver Gould

Gould O (1), de Lacy Costello (1), Francis N (2), Boulind C (2), Beesley K (2), Covington J (3), Ratcliffe NM (1),

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(2) Yeovil NHS Trust, Higher Kingston, Yeovil, BA21 4AT
(3) University of Warwick, Coventry, CV4 7AL

Background

The overarching aim of this project is to improve the efficacy and efficiency of the fast-track pathway for patients referred with suspected colorectal cancer (CRC) by safely reducing the number undergoing negative diagnostic colonoscopy and enabling better utilisation of resources. Non-invasive, easy to use, testing methods are attractive because they support compliance. It is hypothesised that the different VC signature in patients with CRC will enable triaging into those that need invasive colonoscopy and those that can be safely reassured.

3 analytical methods have been compared: selected ion flow tube mass spectrometry (SIFT-MS), field asymmetric ion mobility mass spectrometry (FAIMS), and thermal desorption gas chromatography mass spectrometry sensor system (ATD-GC-MS-S).

Methods

Patients undergoing colonoscopy will be recruited, this project aims to recruit 600 patients a subset of preliminary data for which will be presented herein. From each patient urine sample 4 headspace vials will be filled with 5mL of urine; these vials are then immediately stored at -80°C until analysis, this allows the samples from 1 patient to be analysed on all 3 instruments.

A Voice 200 SIFT-MS was used for analysis; nalophan bags will have ca. 3mL of urine added to them prior to being filled with ca. 1L of zero air. The filled bag is then placed in a 40°C incubator for 30 minutes. A full mass scan is then carried out range from m/z 0-200.

An Owlstone Lonestar FAIMS instrument is used for analysis, the sample is thawed in a 40°C water bath for 30 minutes before being added to the FAIMS sample chamber. For each sample 5 scans are performed.

ATD-GC-MS-S will also be used, as with the FAIMS the sample vials are thawed in a 40°C water bath for 30 minutes prior to loading the tube. The sample headspace is flowed through the tube at a rate of 80mL/min for 2 minutes, the tube is then analysed on the ATD-GC-MS-S.

Here we present preliminary data comparing the outputs from 3 analytical instrument as well as commentary on the usability and reliability of each instrument.
THE STEPHENSON LECTURE ROOM

52. Multimodal breath-based asthma phenotyping using GCxGC-HRTOFMS and SIFT-MS approaches

Pierre-Hugues Stefanuto

16:15


(1) Organic and Biological Analytical Chemistry Group, MolSys, University of Liège, Belgium
(2) ISX and interscience, Breda, The Nederlands
(3) Respiratory Medicine, GIGA I3, CHU Sart-Tilman, University of Liège, Belgium

Background

The ballistic rise of analytical technologies has opened a large playground for all type of “omics” screening, including breath analysis. On one side, separation science based on multidimensional methods such as comprehensive two-dimensional gas chromatography (GC×GC) appeared as one of the methods of choice for the characterization complex mixtures. On the other side, direct introduction instruments such as single ion flow tube mass spectrometry (SIFT-MS) offered the capacity to perform both targeted and non-targeted analyses within a few minutes. In this study, we have compared the complementarity of these techniques for breath-based asthma phenotyping.

Methods

Exhaled breath from 50 asthmatic patients with different inflammatory phenotypes were analyzed by both techniques. As a reference, asthma phenotypes were established using sputum analysis. Breath samples were collected using Tedlar bags. For GC×GC-HRTOFMS analyses, the bags were transferred onto thermal desorption tubes prior to injection. For SIFT-MS, the bags were directly emptied into the instrument. Next, data were analyzed using identical processing workflow.

Results

We observed that both approaches offered similar classification capacities regarding asthma phenotype differentiation. For example, eosinophilic phenotype could be identified with an accuracy ranging from around 80 % for both techniques. In addition, GC×GC-HRTOFMS allowed identifying the putative markers for comparison with previous studies and metabolic interpretation, while SIFT-MS offered a faster screening capacity ideal for population screening.

Conclusions

These two instruments offer an interesting complementarity for the characterization of exhaled breath samples. At the price of high cost equipment and limited adaptability to routine medical usage, GC×GC-HRTOFMS offers the possibility to almost completely characterize a sample composition. This is of prime importance when system biology is considered. For large scale screening, SIFT-MS can generate compositional patterns from direct sample introduction at the same time than other routine medical actions. The accuracy achieves through these two orthogonal platforms underline the robustness of breath-based asthma phenotyping.
## WEDNESDAY 11 SEPTEMBER 2019

### THE TURING EXHIBITION SPACE

<table>
<thead>
<tr>
<th>Start</th>
<th>Finish</th>
<th>Title</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>09:20</td>
<td>Real-time therapeutic monitoring of valproic acid in exhaled breath</td>
<td>Kapil Dev Singh</td>
</tr>
<tr>
<td>09:20</td>
<td>09:40</td>
<td>The Breath Biomarkers Of Tuberculosis Using Model Macaque Monkey</td>
<td>Mohammad Sharif Khan</td>
</tr>
<tr>
<td>09:40</td>
<td>10:00</td>
<td>A Clinical Breathomic Workflow For Metabolic Phenotyping</td>
<td>Dahlia Salman</td>
</tr>
<tr>
<td>09:45</td>
<td>11:45</td>
<td>Focus groups</td>
<td></td>
</tr>
<tr>
<td>11:45</td>
<td>12:15</td>
<td>Reflection Panel</td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>12:30</td>
<td>Prizes and closing comments with announcement of new officer(s)</td>
<td></td>
</tr>
</tbody>
</table>
Real-time therapeutic monitoring of valproic acid in exhaled breath

Kapil Dev Singh

09:00

Singh K (1,2), Ziesenitz V (1), Usemann J (1), Frey U (1), Van den Anker J (1), Datta AN (1), and Sinues P (1,2)

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(2) Department of Biomedical Engineering, University of Basel, Basel, Switzerland

Background

In the field of medicine, serum concentrations of drugs with a narrow therapeutic window, used to treat seizures, are measured to assure the most efficacious and safe way of treating every individual patient. This form of personalised medicine is called therapeutic drug monitoring (TDM). We have explored the possibility to measure and monitor drugs in exhaled breath (EB) with a suitable real-time analytical method, to perform completely painless and non-invasive TDM for a future clinical application especially in pediatric patients.

Methods

We employed SESI-HRMS to obtain highly resolved EB mass spectra. We then statistically compared these EB mass spectra between patients taking antiepileptic drugs against controls (no drugs), to find potential EB-based bio-markers for drugs. We then trained and tested various regression models to predict serum concentration of drugs using selected features from EB mass spectra. All the data analysis was performed by custom MATLAB scripts.

Results

Our data for valproic acid (VPA), an antiepileptic agent, showed m/z at 115.1118, 132.1384, 143.1065, and 160.1331 to be significantly increased among others in patients taking VPA than controls. We hypothesize that these features correspond to proton and ammonium adduct of C7H14O, and C8H14O2, all of which were previously shown to be elevated in response to VPA. Furthermore, using EB measurements we were able to predict serum concentration of both total (RMSE ~5) and free (RMSE ~1.5) VPA in an independent group of patients (test-set). In the near future, we will perform MS/MS on selected ions to confirm their identity using exhaled breath condensate.

Conclusions

This work is a part of an ongoing study and it is too early to come up with a definite conclusion. However, we would like to highlight the following important findings: 1) It is possible to successfully measure EB in pediatric patients treated with antiepileptic medications; 2) Several differentially abundant ions between controls and epileptic patients for various drugs were visualized (shown for VPA here), but the identity and clinical significance of these ions is yet to be determined; 3) Serum concentration of both total and free VPA can be reasonably predicted via EB-based SESI-HRMS analysis.
The breath biomarkers of tuberculosis using model macaque monkey

Mohammad Sharif Khan

09:20

Khan MS (1), Bobak C (1), Mellors TR (2), Flynn JL (3), Scanga CA (4), Lin PL (3), Azmir J (1), Beccaria M (5), and Hill JE (1)

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(2) Geisel School of Medicine at Dartmouth, Hanover, NH 03755
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Background

TB is one of the world’s top health challenges and the most common causes of infectious disease deaths. More than 2.4 billion people, which is 1/3 of the world population, are infected by Mycobacterium tuberculosis. The current method using sputum needs 2-3 weeks to confirm the TB status. Moreover, 1/3 of TB+ patients especially children and HIV+ patients cannot produce sputum. The infection with M. tuberculosis possesses a volatile signature that was previously “sniffed” by rats to diagnose TB infection (sensitivity, 80.4%; specificity, 72.4%) [1]. Identifying the volatile biomarkers would help us to develop non-sputum based TB diagnosis method. The present study utilizes the macaque model of M. tuberculosis. The main objective of this work is to provide validation of the use of breath as a humane and economical tool for pre-clinical research, particularly in the study of disease pathogenesis, vaccine development, and drug treatment regimen research.

Methods

The breath of 35 macaques was sampled using a previously described method [2] before (n=50) and after (n=131) introduction of a low dose of (3-13 CFU) M. tuberculosis Erdman strain via intra-bronchial installation and confirmed by skin test and a PET-CT scan. The breath volatiles were analyzed by comprehensive two-dimensional gas chromatography with time of flight mass spectrometry (GCxGC-TOFMS). The resulted VOCs features were analysed and modeled using Random Forest (RF), Support Vector Machine (SVM) and Partial Least Square-Discriminatory Analysis (PLS-DA) machine learning algorithm to commonly select a small subset of features. The features were then used to construct the Receiver Operating Characteristic (ROC) curve to find the diagnostic performance of the biomarkers.

Results

Out of >5000 total features detected, 429 breath molecules were found in 80% of pre- or 80% of post-infection samples, and were evaluated based on their ability to discriminate between pre- and post-infection samples. The three different machine learning models commonly selected ~10 molecules (dominated by hydrocarbons, ketones, and aldehydes). The model using those biomarkers was validated with randomly selected 1/3 of the dataset (n=60) with an accuracy of 0.99 and 1 for training and test data, respectively. The selected breath biomarkers could differentiate pre- and post-infection macaques breath sample with an area under the receiver operating curve (AUROC) of >90%.
Conclusions

This result showed that the pre-clinical animal models, macaques, can be used to determine TB infection status using exhaled breath in a relatively non-invasive, reproducible manner, generating insights as well as saving researchers money and limiting the use of lethal experimental assays.

A Clinical Breathomic Workflow For Metabolic Phenotyping

09:40

Dahlia Salman (1), Yaser Al-Khalifa (1), Bo Zhao (2,3), Wadah Ibrahim (2,3), Matthew Richardson (2,3), Robert Fee (2,3), Amisha Singapuri (2,3), Dorota Ruszkiewicz (1), Michael Wilde (4), Rebecca L Cordell (4), Neil Greening (2,3), Salman Siddiqui (2,3), Chris Brightling (2,3), C L Paul Thomas (1)

(1) Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK.
(2) College of Life Sciences, Department of Infection, Immunity and Inflammation, University of Leicester, University Road, Leicester LE1 7RH
(3) Leicester NIHR Biomedical Research Centre (Respiratory theme), Glenﬁeld Hospital, Groby Road, Leicester LE3 9QP
(4) Department of Chemistry, University of Leicester, Leicester, UK

Background

The East Midlands Breathomics Pathology Node (EMBER) MRC pathology node has described a methodology workflow for metabolomics profiling and marker discovery in human breath using thermal desorption-gas chromatography–mass spectrometry (TD-GC-MS. Participants (n=300) with self-reported acute breathlessness were studied across two adults and children acute admission units (1). A workflow for metabolomic phenotyping was proposed to cover the design of sampling and operational SOPs, as well as ethical consideration, breath sampling, data processing, statistical analysis and classification models.

Methods

TD-GC-MS data were aligned and deconvolved using AnalyzerPro software to extract 350-500 VOC features per sample. Each breath sample was divided into four segments and a deconvolution method was optimised per segment to minimise over or under-deconvolution. Deconvolved features were then clustered in groups of VOCs with similar mass spectra and retention index profiles using the VOCCluster algorithm. VOCCluster algorithm, prototyped in python, was created from a heuristic ontology based on the observation of experts undertaking data processing with a suite of software packages.

Results

It was evaluated and demonstrated an accuracy of 96% when the results were against a panel of ground truth compounds and compared to other clustering methods used in previous metabolomics studies such as DBSCAN and OPTICS. Each clustered group was assigned a unique identifier in the form of (BRI- m/z1 - m/z2 - m/z3 - m/z4 - m/z5) and a breath matrix of endogenous, exogenous and methodology artefacts was created within 2 hours for n=74 samples.

Conclusions

Post-processing treatments and multivariate statistical modelling were carried out on the produced breath matrix to test for the presence of significant discriminators between different clinical cohorts. Different classification methods such as logistic regression, elastic net and support vector machine learning models were tested for a panel of discriminatory breath markers. Multi-stage quality control measures were taken throughout the workflow and together with raw and processed data are being archived using the CHARTOOL into the project repository.

References:
THE TURING EXHIBITION SPACE

1. Further Evaluation of a Standardized Breath Sampling Device for Off-line Exhaled Breath Analysis

Harshman SW (1), Pitsch RL (2), Davidson CN (3), Scott AM (3), Strayer KE (1), Hill EM (1), Smith ZK (1), Brothers MC (1), Schaeublin NM (1), Slusher GM (1), Meoli SD (1), and Martin JA (3)

(1) UES Inc.,
(2) The Henry M. Jackson Foundation for the Advancement of Military Medicine,
(3) Air Force Research Laboratory; 711th Human Performance Wing/RHXBC, Wright- Patterson AFB, OH, USA

Background

Exhaled breath bags have been the standard method for off-line breath collection. However recently, the Respiration Collector for In Vitro Analysis (ReCIVA) was developed for versatile exhaled breath collection directly onto adsorptive material. The ReCIVA is designed to eliminate sources of variability associated with off-line exhaled breath collection. While potentially beneficial, very little has been done to characterize the overall performance of the ReCIVA breath sampler.

Methods

All participants were fasting (≥1h) males within our facility. Collections on the ReCIVA were performed, as described by the manufacturer, at 200mL/min for 550mL of lower airway breath except where manual calibrated flow rates were applied. All exhaled breath bags were collected for end tidal breath in 1L ALTEF bags using our established collection protocol. Breath volatiles were concentrated utilizing both Tenax TA and Carbograph/Tenax 5TD thermal desorption tubes. For multiple ReCIVA comparisons, ReCIVA serial #33 and #65 were used. For breathing rate determinations the Breathe+ iPhone app was used to guide participants breathing rates to either high, 15 breaths/min, or low, 7.5 breaths/min. All thermal desorption tubes were analyzed by TD-GC-MS using 70eV EI. Isoprene was quantified from samples using calibration curves determined from a custom isoprene canister (1.10ppm).

Results

The results demonstrate using a custom "in-house" flow calibration on the ReCIVA yields consistent isoprene results among both ReCIVA banks (p>0.3131), TD tube types (p=0.3824 between Tenax TA and 5TD tubes), and between the ReCIVA and standard exhaled breath bags (p=0.1534). Furthermore, comparisons among ReCIVA units #33 and #65 using the "in-house" flow calibrations show comparable isoprene results between both units (p=0.1441). Finally, isoprene values from controlled rate breath show no significant difference between the rates (p=0.6666). However, for experiments comparing ReCIVA units and breathing rates, a significant difference among the Tenax TA and 5TD tubes was observed (p<0.0053).

Conclusions

The results suggest the ReCIVA can provide reliable exhaled data independent of the ReCIVA device and breathing rate. These data support the use of the ReCIVA for widespread breath collections.
2. Better Nontuberculous Mycobacteria Detection Using Breath

Purcaro G (1), Nasir M (2), Kang L (1), Davidson R (3), Nick J (3), Hill JE (1,2).

(1) Thayer School of Engineering,
(2) Geisel School of Medicine; Dartmouth College, Hanover, USA
(3) National Jewish Health, Denver, USA

Background

Nontuberculous mycobacteria (NTM) are well-recognized pathogens in the cystic fibrosis (CF) population and are widely viewed as one of the most challenging infectious complications of the disease. Currently, airway cultures combined with an appropriate clinical presentation are the “gold standard” by which all diagnosis and treatment decisions are based. Yet due to required decontamination procedures, airway cultures suffer from low sensitivity and slow growth (up to 8 weeks). Sensitive and specific markers of NTM in the airway are needed.

Methods

Sputum samples were collected from 19 CF patients, and the volatile molecules present in the headspace of these sputum samples were concentrated using HS-SPME and analyzed via comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry GC×GC-TOFMS.

Results

Collectively, the headspace of sputum samples contained over 400 features. Of these, 26 were statistically different between culture-positive and culture-negative samples. From these 26 features, 12 were selected by machine learning algorithm, random forest, to generate a “sputum VOC metabolites expression score”. The change in score of 6 CF patients before and after treatments showed correlation with their culture status.

Conclusions

In this pilot study, using sputum samples collected from 19 CF patients, 12 features were selected to generate a "VOC score". During patient treatment, the "VOC score" showed correlation with patient’s clinical status. With the estimated 840 total samples coming for this project, we are expecting to detect volatile metabolites as culture-independent markers to improve diagnosis and treatment monitoring of airway NTM.
3. **Volatile Molecular Profiling of Floral Odors from Amorphophallus titanum using TD-GC×GC-TOFMS**

Kang L (1), Coyne K (1), Khan MS (1), Hill JE (1)

(1) Thayer School of Engineering; Dartmouth College, Hanover, USA

**Background**

Amorphophallus titanum, commonly known as the corpse flower, is one of the largest and rarest inflorescence plants in existence. The plant is known for emitting a rotting animal-like smell during its peak blooming time, which typically occurs every 3-7 years for only 24-36 hours. The first half of the blooming event, the female flowering phase, releases the most intense odor which fades out during the second half, the male flowering phase. The odor released during the female flowering has been previously studied, yet comprehensive analyses of the volatiles released over time are needed.

**Methods**

The A. titanum plant grown at Dartmouth College’s Greenhouse (Hanover, USA) bloomed in November 2018 and 16 odor samples were collected over the flowering event. Volatile compounds emitted from the plant were absorbed directly to the thermal desorption tubes from the top of the spathe. The volatile molecules in the sample tubes were then thermally desorbed and analyzed via comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry GC×GC-TOFMS.

**Results**

588 volatile features were detected in the thermal desorption samples of the plant. 18 of them were statistically different between before and female flowering. 98 features were statistically different between female and male flowering. These features were further identified based on mass spectral matching and classified based on their chemical properties and correlations.

**Conclusions**

Volatile compounds released by an Amorphophallus titanum plant contains a greater number and wider variety of chemical compounds that differentiate the flowering phases. While the precise identities of the volatile compounds released during each flowering phase remain undetermined, the novel compounds reported in this study represent potential candidates.
4. Technical validation of breath analysis by eNose in disease diagnosis: Tidal breathing vs. vital capacity maneuver

R. de Vries (1,2) Y. Dagelet (1), P. Brinkman (1), N. Farzan (2), Maitland-Van Der Zee AH (1), F. De Jongh (3), E. Haarman (4), H. In 'T Veen (5), P. Baas (6), A. Lucas (7), P. J. Sterk (1)
(1) Amsterdam University Medical Centers, University of Amsterdam - Amsterdam (Netherlands)
(2) Breathomix - Reeuwijk (Netherlands),
(3) Medisch Spectrum Twente - Enschede (Netherlands),
(4) VU Medical Center - Amsterdam (Netherlands),
(5) Sint Franciscus Gasthuis en Vlietland - Rotterdam (Netherlands),
(6) Netherlands Cancer Institute - Amsterdam (Netherlands),
(7) Diagnostiek voor U - Eindhoven (Netherlands)

Background

A variety of techniques to measure molecular profiles in exhaled breath are available. The methods used for breath sampling (e.g. tidal breathing and vital capacity (VC)) vary between different devices. To provide a fundament for standardization, the influence of various sampling techniques on breathprints needs to be examined. The aim of this study was to determine the diagnostic accuracy of breath analysis by electronic nose (eNose), using tidal breaths or a VC manoeuvre, for the diagnosis of asthma, COPD, lung cancer and healthy controls.

Methods

This was a multi-center observational study in controls and asthma, COPD and lung cancer patients. As part of spirometry, breathprints were collected in duplicate by eNose (SpiroNose) [De Vries ERJ 2018]. Subjects performed 5 tidal breaths followed by an inspiratory capacity maneuver, a 5 second breath hold and a slow expiratory VC. Data analysis involved signal processing, ambient air correction and statistics based on principal component (PC), linear discriminant and ROC analysis.

Results

Breath data of 2161 subjects, asthma (n=702), COPD (n=540), lung cancer (n=256) and controls (n=663) were available. The results are presented in figure 1. Conclusions: SpiroNose data obtained by tidal breaths and VC manoeuvres are not interchangeable. VC manoeuvres provide the highest accuracies in the cross comparison of controls and asthma, COPD and lung cancer patients.
Figure 1. A. Exhaled breath analysis by eNose based on tidal breaths; B. Exhaled breath analysis by eNose based on VC manoeuvre. Cross-validation values (%) for the discrimination between healthy controls and patients with asthma, COPD and lung cancer.
5. **Volatile organic compounds in exhaled breath during human endotoxemia**


(1) Department of Pharmacology and Toxicology, NUTRIM School of Nutrition and Translational Research, Maastricht University, Maastricht, The Netherlands

(2) Department of general surgery, Maastricht University Medical Centre, Maastricht, The Netherlands

(3) Department of intensive care medicine, Radboud University Medical Centre, Nijmegen, The Netherlands

**Background**

Systemic inflammatory response syndrome (SIRS) is a life-threatening condition caused by a dysregulated immune response to trauma or infection. To date, SIRS is a clinical diagnosis and there are no clinically used biomarkers to identify this immunological condition. Volatile organic compounds (VOCs) in exhaled breath reflect metabolic changes and may change during the early development of SIRS. The aim of this study is to investigate whether VOCs in exhaled breath can be used as non-invasive biomarker to predict the inflammatory response during human endotoxemia.

**Methods**

We included ten healthy male volunteers. Seven subjects received intravenous endotoxin (2ng/kg Escherichia coli lipopolysaccharide) twice, with a one-week interval. Three subjects served as control group receiving a placebo. At baseline and various time points after endotoxin administration, arterial blood and exhaled breath were collected. The plasma cytokine response was determined using a luminex assay. Breath samples were collected on stainless steel desorption tubes (1TD/Carbopack X) and analyzed by GC-tof-MS. The chromatograms were preprocessed and subsequently analyzed by various linear and nonlinear machine learning techniques. Data were analyzed on individual level, as well as on group level.

**Results**

Analysis of the chromatograms showed uncertain alternations between individuals receiving endotoxin or placebo. Very similar results were obtained for measurements obtained at baseline and various time points post endotoxin administration. Although, no clear differences could be observed, the VOCs that showed the highest differences were tentatively identified as linear- and cyclic alkenes.

**Conclusions**

VOC analysis demonstrated vague differences between individuals injected with endotoxin and those with placebo. These results suggest that injection with endotoxin does not alter the composition of exhaled breath significantly.
6. **Volatile Organic Compounds in Idiopathic Pulmonary Fibrosis: A cross-sectional analysis.**

Hayton C (1, 2), Terrington D (3), White I (1), Ahmed W (1), Vekaria K (1), Wilson AM (3), Chaudhuri N (2), Leonard C (2), Fowler SJ (1, 2)

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(2) North West Lung Centre, Manchester University NHS Foundation Trust, Manchester, UK

(3) Norwich Medical School, University of East Anglia, Norwich, UK

**Background**

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease associated with high morbidity and mortality. The cause is unknown and it predominantly affects male patients aged over 70. Prognosis and progression is extremely variable within the disease cohort. Lung function parameters, such as forced vital capacity, are traditionally used to assess severity and monitor progression, however these lack specificity. There is a need for novel biomarkers which can accurately predict disease outcomes. Previous work has identified four volatile organic compounds (VOCs) which discriminate between IPF patients and healthy controls.[1] The aim of this study was to determine if VOCs can discriminate between different severity stages in IPF patients.

**Methods**

Patients were recruited from a new patient tertiary lung clinic. Inclusion criteria were age over 18 and a multi-disciplinary team (MDT) diagnosis of IPF. Patients were excluded if they had an additional significant respiratory co-morbidity, were a current smoker or had a recent lower respiratory tract infection. Clinical and lung function data were collected and patients completed two breathlessness questionnaires. The GAP index, a validated staging score based on demographic and physiological parameters,[2] was calculated. Exhaled breath samples were taken using the Owlstone ReCIVA device and Tenax GR thermal desorption tubes. Samples were analysed using thermal desorption/gas chromatography-mass spectrometry (TD/GC-MS).

**Results**

67 patients were recruited (53 males). 1 patient was subsequently withdrawn as their diagnosis changed. Patients were divided into three severity groups based on the GAP index. Targeted analysis was performed for the four VOCs (isoprene, p-cymene, ethyl benzene, acetoin) which have previously been reported to discriminate between patients with IPF from healthy controls. Provisional analysis from a subset of 12 patients confirmed the presence of these VOCs in the breath samples of IPF patients in this cohort. No significant differences were observed between severity groups, although there was a trend towards smaller peak areas for all four VOCs in patients in the most severe group. Further analysis is ongoing.

**Conclusion**

Provisional targeted analysis confirms the presence of four previously reported discriminatory VOCs in the breath of patients with IPF. Analysis of the full data set is continuing, to determine if these VOCs correlate with disease severity.

7. Can exhaled breath analysis be used for monitoring the metabolic impact of diet?

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(2) Division Gastroenterology-Hepatology, Department of Internal Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands
(3) Nutricia Research, Utrecht, The Netherlands

Background

Since ancient times, physicians valued human breath as a window of diseased and healthy organs. Thousands of volatile organic compounds (VOCs) are produced in different organs which are transported by blood to the lungs where they are released. Inflammatory and deviant metabolic processes change the composition of these compounds which can be of use for clinical diagnosis and disease monitoring. Many VOCs are also produced by intestinal microbiota. Some of these compounds are excreted into feces while others enter the systemic circulation where they can be further modified by the host. Exhaled breath analysis has been demonstrated for disease monitoring and diagnosis but also for investigating effect of various diet.

Methods

In the two studies exhaled breath was used to monitor the effect of either formulae milk or prebiotics. In the first study, exhaled breath was collected from a group of 29 healthy, non-smoking adult males before ingestion of a study product (baseline measurements, T0) and at the following time points after the test meal: 30, 60, 120, 180 and 240 min. The test meal consisted of two different formula milks: (i) a concept infant milk formula with large, phospholipid coating of lipid droplets (further described as “active”), and (ii) a commercially available formula milk characterised by smaller lipid droplets, further described as “control”. In the second study, fifty-two young adults and 48 elderly consumed 15g/day sugar beet pectin or maltodextrin for four weeks. Before and after the intervention period, exhaled breath samples were collected. Exhaled breath samples were analysed by Gas Chromatography coupled with Mass Spectrometry analysis. In both cases different machine learning technique were used to find differences in volatile compounds.

Results

The statistical analysis by means of regularised MANOVA showed statistically significant differences between active and control formula milk, 240 min after consumption of the product (p-value<0.0001). Interestingly, no significant changes between active and control product at any earlier time points were found. A set of eight VOCs in exhaled breath was statistically significant at 240 minutes between the two formulas. The set of ten VOCs in exhaled breath was found to be statistically different between baseline and the two formulae at T240 (p-value <0.0001.) In case of pectin intervention, tree-based technique has led to classification model with prediction performance of random classifier. This indicates that pectin supplementation did not significantly alter the content of exhaled breath in in elderly or young adults.

Conclusions
The Babbage Room

The analysis of exhaled breath was suitable to monitor metabolic effects after ingestion of infant formulae with different lipid structure. However, following four weeks of pectin implementation did not affect the content of exhaled breath.
Online monitoring of exhaled propofol by dopant-assisted photoionization positive IMS coupled with negative photoionization IMS for rapid measuring propofol concentrations in plasma undergoing orthotopic liver transplantation

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Background

For online monitoring of exhaled propofol, we built a dopant-assisted photoionization positive IMS (DAPI-IMS) (Dandan Jiang et al, Analytica Chimica Acta, 2018), the selectivity and sensitivity of propofol was improved using toluene as the dopant, which could eliminate the interference of sevoflurane in the breathing circle. However, many pharmacological factors such as initial distribution and pulmonary uptake may alter the correlation between breath and plasma, simple and rapid propofol concentration measuring in blood/plasma is necessary. We also built the negative photoionization IMS (NP-IMS) (Xin Wang et al, Scientific Reports, 2016), it can be used in clinical intravenous anesthesia. During orthotopic liver transplantation (OLT), the performance error of TCI was large, especially in anhepatic phase. Therefore, this study aims to monitor breath and blood/plasma propofol concentrations using DAPI-IMS combined with NP-IMS in OLT.

Method

Two patients presenting for OLT were involved. Induction: midazolam 0.05mg/kg, fentanyl 5µg/kg, etomidate 0.3mg/kg and cisatracurium 0.2mg/kg. After intubation, propofol TCI Marsh model, remifentanil TCI Minto model was managed. The sampling tube from DAPI-IMS with a T-piece was connected between the tracheal catheter and the breathing circuit, at a speed of 1 L/min in side-stream mode. Blood samples (1mL) were obtained every 15-30min during anesthesia, 20µL blood sample (aliquots of the supernatant) was injected into a 200 mm × 4.6 mm i.d. C18 silica gel column (Kromasil ODS, 5 µM) for detection of propofol concentration by NP-IMS.

Patient one:

40 years old, hepatolenticular degeneration. After intubation, propofol concentration (Cp) 2.5µg/ml, was administered until the end of operation.

Patient two:

35 years old, chronic hepatitis and cirrhosis, After intubation, propofol concentration (Cp) 2.5µg/ml, was administered until the beginning of operation, and then increased to 3.5µg/ml until anhepatic phase. During anhepatic phase Cp was 2.0µg/ml and increased to 3.0µg/ml during neohepatic phase. To fit the anesthetic depth we adjust the target propofol concentration according to BIS indexes, which were maintained between 40-60.

Results:

In case one, when Cp was 2.5µg/ml, plasma and blood propofol concentration got the peak of 8.4µg/ml and 6.4µg/ml after ten minutes infusion, while first exhaled propofol peak was 7min later. Exhaled and blood/plasma concentrations both increased in anhepatic phase, and even a time interval before anhepatic phase, which may suggest a
potential reduce of liver blood flow. The profile of propofol in breath was smoothly altered in accordance with blood/ plasma concentration. The correlation between exhaled propofol and blood/plasma propofol concentration was found ($r^2=0.71$, $r^2=0.71$, $n=25$).

In case two, at 5 min before anheptic phase, there was a sharp decrease of end tidal carbon dioxide partial pressure (PetCO2) from 35 mmHg to 19 mmHg while partial arteria blood pressure of carbon dioxide (PaCO2) was increased in blood, followed by a decrease to 93% of pulse oxygen saturation (SpO2). We noticed the surgeons the possibility of lung embolism and hemodynamic indexes recovered 5 min later after symptomatic treatment. During the anhepatic phase of this case, exhaled propofol did not increased as propofol concentrations in blood/plasma, with a lower level contrasted with that in paleohepatic phase. Dissatisfactory correlation between exhaled propofol and blood/plasma propofol concentration was found ($r^2=0.45$, $r^2=0.26$, $n=18$).

Conclusions:
DAPI-IMS is a suitable method for online monitor of exhaled propofol, and NP-IMS promises a rapid method for plasma propofol concentration monitoring which help anaesthetists achieve an accurate anaesthesia, especially in critically ill patient. The increase of exhaled propofol during anhepatic and pre-anhepatic phase indicated a potential ischemia of liver in normal patient, while in suspicious pulmonary embolism patient, exhaled propofol decreased simultaneously with PetCO2. This may become an early warning of adverse event.
9. **HS-GC-IMS as a simple and automatic tool for clinical assessment of propofol concentrations in human plasma**

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Background

The physicochemical properties of propofol result in a measurable amount of propofol in gases in close proximity to the surface of hydrophilic solutions. Combining IMS with headspace GC is a more suitable and elective method to make better use of its advantages and produce better analysis results. Therefore, we aim to explore a new approach for propofol concentration monitoring using HS-GC-IMS.

Methods

The testing plasma was drawn from 6 healthy volunteers. A plasma stock with 100 μg/mL propofol was prepared by weighing and dissolving the propofol in the unspiked plasma, and then mixed with a liquid mixer for 1 min. The plasma samples with lower propofol concentrations were obtained by diluting the plasma stock with unspiked plasma. All blood and urine samples were analyzed with a HS-GC-IMS instrument (FlavourSpec®) from G.A.S. (Ge Gesellschaft fur Analytische Sensorysteme GmbH, Dortmund, Germany), equipped with an autosampler unit (CTC Analytics AG, Zwingen, Switzerland) that can be directly sampled from the headspace by using a 1mL syringe. GC was fitted with a 15m gas chromatographic column (FS-SE-54-CB-1, ID: 0.53 mm) to separate volatile components and coupled to IMS. The data was acquired in positive mode and analyzed using LAV software (version 2.2.1) from G.A.S.

Results

The drift time of propofol was constant at 10.72ms and retention time 1688.82s. The calibration curve for propofol was linear in the range of 0.5 to 10μg/mL (clinical range) with a correlation coefficient R2 of 0.993, the repeatability was found to be satisfactory for the measurement of propofol by HS-GC-IMS, with a relative standard deviation (RSD) of 2.0%~9.1% for 5 times measurement one day, and 6.9~12.1% in continuous 5 days, in 1.0, 5,10μg/mL concentration separately. Finally, the plasma propofol concentration was measured by HS-GC-IMS in a patient undergoing target controlled infusion anesthesia and propofol concentration were well analyzed without the effect of other volatile organic compounds in plasma.

Conclusions

This is a pilot study for using HS-GC-IMS in propofol concentration detection during anesthesia. Due to the non-preprocessing of the plasma and automatic sample injection system, HS-GC-IMS may offer a simple and automatic method to estimate plasma propofol concentration in clinic, especially for large sample research.
10. GC-IMS (Gas Chromatography - Ion Mobility Spectrometry) for VOC breath analysis profiling

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Background

Current identification and concentration analysis of Volatile Organic Compounds (VOCs) in breath, with an acceptable accuracy, are performed with Mass Spectrometer based techniques, e.g. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), Proton-transfer-reaction mass spectrometry (PTR-MS) and Gas Chromatography Mass Spectrometry (GC-MS). There is a need for more affordable and portable alternative technologies which are more suited to point of care environments.

Methods

Here we propose GC-IMS as an alternative option for breath VOCs analysis. The instrument used in these studies has two detectors: a conventional sensitivity Faraday plate electrometer and a single ion detector for use when VOC concentrations are below ppt range. We present the results on GC-IMS VOC profiling for an in vitro study of CALU-1, a non-small cell lung cancer (NSCLC) cell line as well as K562, a chronic myelogenous leukemia cell line. In addition, the current technology was tested in preliminary clinical studies for a number of viral and bacterial infections.

Results

It has been shown in the in vitro studies that GC-IMS with Machine Learning (ML) algorithms enables clear identification of CALU-1, and K562 cells produced VOC profiles. The preliminary clinical study results obtained also show a great potential for VOC profiling of bacterial and viral infections in clinical environments. We believe that this GC-IMS method, with the single ion detector, can provide a comparable, if not improved resolution, detection time and sensitivity than other MS based technologies.
11. Analysis of temporal variance patterns in online proton transfer reaction – time of flight – mass spectrometry data from the exhaled breath of smokers

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Background
Real-time information obtained during direct analysis of exhaled breath using proton transfer reaction – time-of-flight – mass spectrometry (PTR-ToF-MS) contains information that is not currently being utilized in the discrimination of cohorts of individuals.

Methods
We propose a novel method for the interrogation of individual breath samples with respect to time, identifying and quantifying the temporal behaviour of PTR-ToF-MS mass channels during a tidal breath sample via the use of bivariate correlation statistics and a breath analogue as a reference point. The aim of this methodology is to improve the classification of groups of individuals as a method of discovering biomarkers of interest via data-driven variable pre-selection based on the temporal information available from PTR-ToF-MS data. This novel method was then demonstrated using an example dataset of 266 individuals who provided sixty seconds of tidal breath into a PTR-ToF-MS and information on their smoking histories. Two linear discriminant analysis (LDA) models were designed using the measured intensities from the instrument, with one that included the temporal correlation information as a pre-processing step and another that did not include this information.

Results
Inclusion of temporal correlation information provided an increase in the model’s ability to accurately identify current smokers, ex-smokers and non-smokers from each other from 48.5% to 79.7% of accurate classifications generated by the model. Six mass channels were identified as containing discriminatory information from the model including the temporal information as a part of the workflow. Incorporation of the temporal correlation coefficient into the data analysis workflow for online PTR-ToF-MS data improves the classification performance of LDA significantly when used in tandem with intensity values.

Conclusions
This work demonstrates the additional discriminatory power that is contained within the temporal dimension, warranting further utilisation in studies with real time mass spectrometric techniques.
12. Volatile profiles of MSTO-211H malignant mesothelioma cells.

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Background
Malignant mesothelioma (MM) is an incurable cancer usually associated with previous exposure to asbestos. Diagnosis is traditionally at a very late stage leading to poor prognosis and limited treatment options. Volatile organic compound (VOC) biomarkers in breath have been used to discriminate MM patients from other groups, possibly aiding in non-invasive diagnosis. Headspace analysis of MSTO-211H, a biphasic MM cell line derived from metastatic pleural effusion, was used as a MM breath analysis model to identify endogenous VOCs released in vitro.

Methods
Headspace analysis was performed on MSTO-211H cell cultures and cell-free RPMI media controls. A solid-phase microextraction (SPME) fibre (Supelco, DVB/CAR/PDMS) was exposed to the headspace of culture vessels and VOCs extracted at 37°C for 20 minutes. The fibre was desorbed for 1 minute at 250°C and specific VOCs identified through gas chromatography-mass spectrometry (GC-MS).

Results
Preliminary data showed that the most prominent VOCs identified in cell-free RPMI samples were styrene, decane, dodecane and tetradecane. The composition of this VOC profile was altered in MSTO-211H cell cultures - further method development is required to identify the subtle changes in specific VOCs.

Conclusions
MM cell line headspace analysis provides a more certain origin of VOCs release, with the most appropriate biomarkers identified both in vivo and in vitro. Further experiments are required comparing VOC profiles of different MM cell lines and non-malignant cell types to identify additional candidate biomarkers. In vitro headspace analysis can act as a model for the study of MM diagnosis with further experiments involving oxidative stress and cell line genomics exploring the origins of VOC production.
13. Investigation of Volatile Compounds Produced by Pathogenic Oral Bacteria

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Background

Infections by oral pathogens are one of the most common health problems worldwide. Due to the intimate connection between exhaled breath and the oral cavity, breath analysis could potentially be used to diagnose oral infections. Comprehensive studies about volatile emissions by the oral pathogens causing gingivitis and periodontitis are still scarce, and most of the earlier research has mainly focused on volatile sulphur compounds and their connection to halitosis.

Methods/Results

In our recent study, we have performed in vitro headspace measurements on four important oral bacterial species (P. gingivalis, T. forsythia, P. intermedia and P. nigrescens) using proton transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS). The studied oral bacteria produced abundance of substances, however, we considered 13 compounds produced to be most relevant as potential biomarkers. Seven of these species were tentatively identified as hydrogen sulphide, methanethiol, acetone, dimethylsulphide, isoprene, cyclopentanone and indole. For the remaining six signals the assignment was less certain, because the PTR-TOF-MS method does not provide specific structural information about the mass signals. Therefore, distinguishing between isobaric species and structural isomers is impossible. Further analysis of the unidentified signals with GC-MS is one of our main interests in the future.

Conclusions

In our study, we found that some of the oral bacterial species can be separated from each other according their volatile fingerprints. These results can hopefully be used in the future development of both bacterial screening as well as breath analysis for oral infections. In addition, several of the detected compounds are known to be toxic, which points to an intriguing possibility of studying the connection between the bacterial virulence and the emitted volatile compounds. The possible effects of microbial volatiles on oral tissues and their destruction during periodontitis is especially interesting. In the next stage of our research, we also aim to extend our measurements to other oral bacterial species and mixed cultures.
14. Short and long term variability of volatiles in oral and nasal breathing analysed by selected ion flow tube mass spectrometry

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Background
Breath based non-invasive diagnostics have the potential to provide valuable information about a person’s health status. However, it is not yet widely used in clinical practice due to multiple factors causing variability and the lack of standardized procedures. This study focuses on the comparison of oral and nasal breathing and on variability of VOCs over short and long term.

Methods
Selected ion flow tube mass spectrometry (SIFT-MS) was used for on-line analysis of selected volatile organic compounds (VOCs) in oral and nasal breath of 10 healthy individuals 5 times in one day and 6 times spread over 3 weeks. Intra-class correlation coefficients (ICC) were used to assess short and long term variability. Additionally, ambient air was analyzed at 60 of the 93 breath sampling timepoints. The selected VOCs common in exhaled breath were 1-propanol, 2,3-butanedione, acetaldehyde, acetone, ammonia, benzene, dimethyl sulfide, isoprene, pentane, propanal and styrene.

Results
VOC levels in ambient air were not correlated with those in exhaled breath. Furthermore, all VOCs were significantly higher in breath samples compared to ambient air. Most VOCs were significantly higher in oral breath compared to nasal breath, except for acetone, propanal, dimethyl sulfide and ammonia. Reproducibility varied depending on the VOCs. Most physiologically relevant VOCs (acetone, isoprene, 1-propanol, acetaldehyde, ...) showed good to very good reproducibility (ICC > 0.61) mainly in oral breath and over a short term period of one day. Both in oral and nasal breath factor analysis showed 3 main clusters: 1-propanol, dimethyl sulfide and 2,3-butanedione; isoprene; and ammonia.

Conclusions
Real-time SIFT-MS analysis is a reliable method to assess variability of breath VOCs and ambient background. This method can be further optimized by calibrating the instrument which is important for exact quantification. However, in comparative studies such as ours it is not strictly necessary.
Noisy breathing infants: better phenotyping with breath volatiles?

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Background – Noisy breathing is very common in infants and young children. Discriminating between different phenotypes, like “wheeze” and “rattle”, is crucial to initiate the correct treatment. However, mislabeling of these breath sounds and the lack of an objective method for diagnosis could lead to under- and overtreatment. This study aims to objectively differentiate between different noisy breathing phenotypes based on non-invasive sampling methods.

Methods – Twenty-three patients were recruited at the pediatric practice. Treatment and follow up (3 visits, every 3 weeks) did not deviate from the standard clinical procedure. At every visit the pediatrician examines the child and scores the noisy breathing (wheeze and rattle) based on observation and auscultation. Additionally, exhaled breath (using ReCIVA® (Owlstone Medical, UK)) and a nasopharyngeal swab (NPS) are collected. Exhaled volatiles are analyzed using selected ion flow tube mass spectrometry (SIFT-MS) with the Syft Voice200 (Syft Technologies, New Zealand). SIFT-MS data were preprocessed by subtracting 3 times the standard deviation of 25 blanks. Features that were present in less than 20% of cases and of which the maximum was lower than 15 counts per second were excluded. The NPS is used to analyze 10 nasal mucus inflammation markers using an electrochemiluminescence sandwich immunoassay (Mesoscale Discovery, USA) and to detect the presence of respiratory pathogens by PCR.

Results – After preprocessing of SIFT-Ms data 500 features remained, which were reduced (by factor analysis) to 6 breath factors. A second factor analysis was performed on the combined data of the breath factors, the nasal mucus inflammation markers, the respiratory pathogens and the pediatrician’s noisy breathing score. This revealed that the rattle score was most was most correlated with the breath factors of the previous factor analysis. Discriminant analysis confirmed these findings and was able to classify 70.96% of patients correctly in the correct noisy breathing class (strong wheeze, strong rattle, moderate rattle, mild rattle, recovered).

Conclusion – The pediatrician’s rattle score based on auscultation was more correlated with breath volatiles than with nasal mucus inflammation markers and the number of respiratory pathogens present. These preliminary results indicate the potential of exhaled breath volatiles in the differential diagnosis of noisy breathing.
16. Online Monitoring of Exhaled Acetone by Stand-alone Single Photon Ionization Ion Mobility Spectrometry

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Background

As a biomarker in the metabolism, acetone is closely related to various physiological phenomena such as diabetes, fasting and exercise. From a clinical perspective, the realization of online accurate quantitative measurement of acetone in exhaled air is of great significance for the early clinical diagnosis and follow-up treatment of related diseases. Therefore, detecting the concentration of acetone in the exhaled breath is helpful to provide preliminary information for the health of the human body.

Methods

With high sensitivity and fast analysis speed, ion mobility spectrometry (IMS) has been widely used in the field of rapid measurement. However, the radioactivity of 63Ni ionization source limits its application due to safety, environmental, and regulatory concerns. Moreover, high moisture influenced seriously the online monitoring of acetone in positive ion mode. To continuously monitor the exhaled acetone, a single photon ionization ion mobility spectrometry (SPI-IMS) coupled with online semiconductor cooling apparatus was applied for the measurement of acetone in exhaled air without any other sample pre-separation.

Results

At the optimized conditions, the linear response range for breath acetone was achieved to be 20 ppbv to 1 ppmv with a limit of detection (LOD) of 1 ppbv. This method was used for online monitoring the exhaled acetone of volunteers and patients during surgery. The influence of moisture and other interferents in exhaled breath were effectively eliminated via the online cooling and selective photoionization method.

Conclusions

A SPI-IMS combined with online semiconductor cooling inlet was developed for monitoring of acetone in exhaled air, which can provide information for the diagnosis of diseases such as diabetes and physiological phenomena such as exercise fat reduction. The combination of semiconductor cooling technology and SPI-IMS could effectively eliminate the influence of moisture in the exhaled air.
17. Testing and customization of the ReCIVA™ breath sampler

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Background: Currently we use a self designed reservoir breath sampler to load Tenax tubes for GCMS analysis. As the ReCIVA breath sampler is easier to handle, more mobile, and now broadly used in breath studies, we tested this device and evaluated its fit for our purpose.

For studies with immunocompromised patients the ReCIVA mask design is not optimal as the nose is very close above the unsterile adsorption tubes. The mask design also increases the risk of tube contamination and inhalation of absorption material. Therefore we designed a connector tube with dimensions that fits into the ReCIVA and holds the adsorption tubes which are separated from the subject by a sterile filter with mouthpiece.

Methods: We first compared our reservoir sampler with standard ReCIVA setup. Next we tested the variability between the 4 simultaneously loaded tubes. After initial tests we decided to run the ReCIVA in the "upper and lower airway"-mode, which is similar to our reservoir sampler. The standard setup was then compared to the setup using our adaptor and a sterile filter.

Results: In line with the fact that our reservoir sampler loads 2.5 L of breath to each tube compared to 500 mL loaded by the ReCIVA we generally found lower levels of VOCs with the ReCIVA, but overall a high correlation between samplers. Larger deviations are likely linked to material related VOCs. The variability between tubes loaded by the 4 ports of the ReCIVA was low. Slightly lower overall breath VOC levels compared to the mask were detected using our novel connector tube. An improved 3D-print tube design with a better sealed tube fit improved this. Comparison of data from the "peppermint oil"-trial showed similar wash out curves for our reservoir sampler and the ReCIVA, both with the mask and the novel tube connector setup.

Conclusions: Based on our data, we consider the ReCIVA as a valuable tool in breath research. Our novel connector design shows comparable results to the mask setup and could be a useful supplement to the ReCIVA system.
Background

An open wound can be thought of as its own mini microbiome, providing a readily available nutrient source for a variety of opportunistic microbes. Efficient monitoring of open wounds is critically important to prevent severe infections. The detection of volatile organic compounds (VOCs) emitted from pathogens colonising the wound bed can potentially be used to diagnose infections early based on the resulting VOC profile of the wound sample.

Methods

S.aureus and P.aeruginosa liquid cultures were grown in headspace vials. These pathogens are two of the most prevalent bacteria responsible for wound infections. Solid Phase Microextraction (SPME) fibers collect VOCs from the headspace of the bacterial samples following incubation. The fiber chemistry is carboxen/polydimethylsiloxane, which has a high affinity for small, highly volatile compounds. Sample analysis was carried out using GC/MS.

Results

Significant variation in VOC emission was observed in bacterial samples over time. Bacteria emit species specific VOC compounds. Acidic compounds such as isovaleric acid and acetic acid were only detected in S.aureus samples, while compounds such as 2-nonanone, 1-undecene and 1-butanol were only found in P.aeruginosa samples.

Conclusions

Common pathogenic bacteria can be identified based on their VOC emissions. Time taken between sample collection, sampling, and analysis will be a critical factor to address when examining real wound samples.

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Background
Malignant pleural mesothelioma (MPM) is an aggressive cancer, with a five-year survival rate of <5%. Therapy is palliative, including different combinations of chemotherapy, surgery, radiation and immunotherapy. Therapeutic response is monitored by CT scans using the modified RECIST criteria. Exhaled breath is easy to retrieve and contains volatile organic compounds (VOCs) that reflect metabolic processes in the human body, making it suitable to determine disease state. Recently, breath analysis has proven to be useful for MPM diagnosis (Lamote et al, 2017, Eur Respir J), allowing its exploration to monitor therapeutic response non-invasively.

Methods
Our aim is to explore the possibility of monitoring therapeutic response by comparing VOCs in breath of treated and untreated mesothelioma patients. For this pilot study, breath samples were obtained from 15 untreated and 15 treated MPM patients. VOCs in the breath samples were analysed using a multicapillary column/ion mobility spectrometer (MCC/IMS).

Results
The ROC curve of the model for discriminating treated from untreated patients shows a good discrimination between these two groups with an accuracy of 83% and area under the ROC curve of 0.904. A clustered heatmap and PCA plot were also constructed, showing a relatively good separation as well between the treated and untreated patients.

Conclusions
A significant difference in breath profiles was observed between treated and untreated patients (83% accuracy). Further research will determine if this difference is the result of tumor reduction, the presence of antitumoral agents or changes in inflammatory response. Comparisons with CT scans will also be made to see if there is a correlation with the clinical response rate.
20. Testing a Commercial Vapour Generator to Calibrate Analytical Instrumentation for Targeted Breath Research

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Background
Breath analysis is a promising technique for potential disease biomarker studies, however, analytical equipment is subject to drift, particularly after prolonged use. Hence, there is a need for an easy-to-use, vapour generator, to generate calibrant mixtures in high humidity environments for evaluating detection devices. This work addresses the challenge of assessing the operational integrity and accuracy of analytical instrumentation.

Methods
A handheld vibrating mesh medical nebuliser (Omron Micro Air NE-U22V) was utilised to generate low concentrations (ppb-ppm range) of volatile compounds linked to human metabolism from aqueous solutions of p-cresol, 1-propanol and 2-butaneone. The aerosol mist produced was coupled to a VOICE 200 SIFT-MS for quantitative analysis.

Results
The results showed that aqueous solutions of a robust panel of potential biomarkers, p-cresol, 1-propanol and 2-butaneone, were able to generate low vapour concentrations in the ppb-ppm region. There is potential to nebulise multiple compounds simultaneously which may be useful in testing targets vs. interferents to assess detector cross sensitivity. SIFT-MS output demonstrated good reproducibility and repeatability of the method for instantaneous vapour generation. This method is applicable to a wide range of compounds many of which it is difficult to obtain gas standards or other standards for at low concentrations.

Conclusions
The analysis generated good results detailing how nebuliser technology can indeed produce low vapour concentrations (ppb-ppm) of disease linked volatile compounds to calibrate analytical instrumentation. Aside from contributing toward breath analysis, the method has potential for uses in other fields such as explosives research where it could generate low vapour concentrations of explosive linked compounds to calibrate field deployed explosive sensors and sensing material.
21. Evaluation of acetone absorption bands for breath analysis in the UV and IR region with optical sensors

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Background

Prior to the development of optical sensors for gas analysis, applicable absorption lines of the target analyte have to be determined. Especially the gas matrix of human breath exhale is rather complex and hence, needs detailed examination regarding potential cross sensitivities and interference suppression techniques for acetone detection. Exogenous substances, e.g. present in ambient air in clinics have been considered, too. The choice of suitable absorption wavelengths or wavelength regions is thus crucial for optical sensor development.

Methods

Literature research has been performed to first determine potential interfering gas species and their abundance in human breath exhale. Subsequently, measured FT-IR data from the PNNL database and a software exploiting the HITRAN databank have been used to determine absorption bands of interest for acetone detection in the infra-red region. Besides, the MPI-Mainz UV/VIS Spectral Atlas has been consulted to obtain data for the cross sensitivity analysis in the UV region. Not only the extent of absorption band interference but also the absorption cross section intensity of acetone was considered.

Results

13 substances have been identified including five exogenous molecules originating from disinfectants and cleaning agents used in the clinical environment to potentially distort acetone detection. In the IR region, the wavenumber sections at 3024cm⁻¹ and 3042cm⁻¹ as well as at 1205.5cm⁻¹ and 1208cm⁻¹ have been identified as the most promising ones. The disinfectants and cleaning agents show high interference potential in the IR region. In the UV region at 35971cm⁻¹, however, only molecules with carbonyl groups like formaldehyde have a considerably interference potential.

Conclusions

To allow sub ppm detection of acetone in human breath exhale in the IR region, cross sensitivity suppression techniques have to be applied since especially the absorption bands of the exogenous substances strongly overlap with the acetone bands. Multiple measurements at different wavelengths combined with linear regression or similar post processing methods might pave the way for reproducible breath acetone measurements in the IR region. In contrast, the absorption peak in the UV region offers an elegant solution to avoid interferences and is recommended to consider as well.
22. Effect of physical exercise on emanation of hydroxyl radical in the skin gas

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Background:
It is well established that physical exercise increases mitochondrial production of hydroxyl radical (OH) in the body. We previously reported that measurement of OH emanated from the skin was achieved by the laser induced fluorescence (LIF) system equipped with a semiconductor laser, a chamber for measurement of skin gas and a detection unit. In this study, we investigated the effects of physical exercise on emanation of OH in the skin gas.

Methods:
Male healthy volunteers performed incremental exercise up to the submaximal loads (80%) and subsequent exercise for 10 min on a cycle ergometer. Skin gas was measured before and immediately after the exercise, and after 1 h rest. In the LIF system, a palm of the subject was placed on the chamber and OH emanated from the skin gas was directly measured.

Results:
The amount of OH in the skin was significantly increased by the exercise and decreased to the baseline level after 1h rest.

Conclusions:
Physical exercise increases emanation of OH radicals from the skin surface. OH in the skin gas can be measured in real-time and high sensitivity by LIF.
23. **Assessing and managing long term analytical stability in TD-GC-MS instruments for breath sampling**

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**Background**

The use of Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS) in breath analysis offers many advantages for biomarker discovery, particularly high sensitivity and the ability to identify features of interest. However, due to the degradation seen after sample storage, analysis must occur regularly and in small batches which can result in a fragmented data set. We aim to determine which tuning parameters are most important for ensuring instrument stability and produce a model of column degradation to help minimise variability and reduce batch-to-batch variation.

**Methods**

As part of routine analysis for several ongoing studies TenaxGR tubes were loaded with a standard mix consisting of 25 compounds in order to monitor analytical performance. These standards were compiled to create a data set that was divided into batches based on analysis date. Analytical instability was assessed based on MS gain and resolution parameters and the intensity of background artefacts produced during thermal desorption. Limits of detection for each compound in each batch were derived and Pearson correlation coefficients were calculated between MS parameters and the intensities of each compound. Gaussian curves were fitted to chromatographic peaks from the standards mix using the Nelder-Mead algorithm in order to assess column degradation.

**Results**

Limits of detection were found to vary between batches. MS parameters correlated with the changes in intensity for the compounds in the standards mix and correlations are discussed in terms of each compounds’ chemical properties. Critical tuning parameters for instrument stability are described. A cut-off point for column degradation is determined from the residuals of the fitted Gaussian model.

**Conclusions**

Assessing and maintaining the stability of TD-GC-MS systems is challenging but is crucial to ensure that the collected data is reproducible and requires minimal treatment during pre-processing for biomarker discovery.
24. Perioperative exhaled breath analysis for distinguishing early gastric adenocarcinoma from advanced gastric adenocarcinoma

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Background

The association between cancer and volatile organic compounds (VOCs) from exhaled breath has attracted increasing attention from researchers. The present study reports on a systematic study of breath profiles of metabolites in human gastric disease by pattern recognition methods. These potential biomarkers which could distinguish early gastric adenocarcinoma from advanced gastric adenocarcinoma were analyzed.

Methods

Breath were collected from 15 early gastric adenocarcinoma patients and 15 advanced gastric adenocarcinoma patients when the patients came into operation rooms, then solid phase microextraction-chromatography-mass spectrometry (SPME-GC-MS) was used to analysis the breath volatile organic compounds. All patients were intestinal preparation with polyethylene glycol on the day before surgery. The statistical methods principal component analysis (PCA) and partial least-squares discriminant analysis (PLSDA) were performed to deal with the final dates.

Results

Nonanal and (3-tert-Butyl-5-hydroxymethyl-cyclohex-2-ethyl)-methanol were found at significantly high levels in the group of advanced gastric adenocarcinoma patients than in the early gastric adenocarcinoma group (P<0.01). PCA showed 8 component, R2X=0.917, Q2=0.593. PLSDA showed 2 component, R2X=0.497, R2Y=0.695, Q2=0.444.

Conclusions

Compared with early gastric adenocarcinoma subjects, patients with advanced gastric adenocarcinoma exhibited a distinct breath metabolic profile with respect to VOCs. The analysis of exhaled breath VOCs appears to have potential clinical applications for distinguishing early gastric adenocarcinoma from advanced gastric adenocarcinoma.
25. Chemical-Based Sensors for the Detection of Organic Compounds

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Background

Detection systems for volatile organic compounds (VOCs) have attracted great interest over the last few years. New developments in sensors and analytical detectors and improvements in their sensitivity has opened up the possibility of portable instruments for disease diagnosis. Volatile organic compounds are also relevant in the diagnosis of some human diseases and monitoring of human metabolism, for example acetone on breath can be related to a ketoacidosis state in diabetic patients or linked to dietary alterations in healthy individuals. However, there is still a need to develop better instruments particularly sensor-based instruments which often suffer from poor selectivity.

Methods

Metal-oxide sensors, in particular, ZrO2 doped with rare earth metals have been shown to be a promising material in order to produce sensors for the detection of acetone. This type of material can, in fact, give a response both from a particular kind of chemiluminescence, called cataluminescence, and from a reversible change in the electrical resistance. This multimodal response sensor has some advantages over conventional metal oxides. Indeed, the cataluminescent response has faster kinetics and a relative insensitivity to humidity compared to conventional metal oxide resistance based sensors. They also typically operate at lower temperatures in the range 200-300 °C.

Results

These types of multimodal sensors have been tested at different temperatures (200-350 °C) and exploring a large number of VOCs, in addition to acetone, in order to map the sensor’s response profile and find the optimum sensitivity and selectivity of the sensor.

Conclusions

These sensors show promise as standalone selective sensors for detecting VOCs relevant to human disease and metabolism. They could also be used in conjunction with gas chromatography instruments to produce GC-Sensor instruments with faster kinetics.
Analysis of breath data with machine learning and pattern recognition

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Background

Human breath is a particularly difficult challenge in elemental analysis. To cope with this difficulty, our proposed analysis strategy will be based on development of suitable algorithms that are robust, accurate and can operate on an advanced form of plasma OES sensor.

We employ Partial Least Squares-Discriminant Analysis (PLS-DA). This is an algorithm applicable in many contexts which specializes in predictive and descriptive modeling along with discriminative variable selection. It is also a recognized technique for handling high dimensionality via latent variables for binomial and multinomial classification of spectra data. Our spectra were obtained from an RF plasma formed in a quartz capillary between two exterior ring electrodes while argon was used to sustain the plasma. The capillary outlet was a large distance (~100 cm) from the plasma to minimise atmospheric impurity back-diffusion and the system was initially conditioned to remove background impurities, over 21 days, using a 100% Argon plasma and exterior IR heating while monitoring spectral impurity bands.

Methods

Firstly, before proceeding with breath analysis, we have investigated supervised learning using Partial Least Squares Discriminant Analysis (PLS-DA) on a gas mixture dataset of He-CH4 spectra where the CH4 concentration varies from 0 – 100 ppm. He/CH4 is a complex gas mixture but is still simpler than external clinical or environmental samples. The data was collected in a matrix of 3648 variables (wavelengths), which form 9 CH4 concentration categories (0, 1, 2, 4, 6, 12, 23, 77, 100 ppm).

Later, we have tried all achieved result into another experiment. In this method, exhaled breath was collected from five participants. Spectra were collected using an Ocean Optics HR4000CG-UV-NIR spectrometer in the wavelength range 194 – 1122 nm (interval 0.25 nm), with a slit width of 5 μm and a minimum optical resolution >0.5 nm.

Results

From a computation perspective, the major difficulty of CH4/He spectra data is the high feature dimensionality, along with temporal instability or drift, collinearity and a high matrix component. These challenges decreased after pre-processing of spectra to include autoscaling, smoothing and baseline correction, followed by data segmentation, VIPs selection and peak concatenation. As a result, spectral features corresponding to helium, carbon, hydrogen and impurities (N, O, OH/H2O) were observed and the algorithm accuracy on this data improved to 98% with < 15 LV.

It seems when evaluating breath, it is critical to look at the change in signal found in the plasma argon. Significantly, wavelength trend, peak width and location were identical for all participants except one, where movement in wavelength’s interval ~ 700-800 nm was found, and this can be taken as evidence of the change in argon signal. In contrast, as proved in previous experiment, the fluctuation of intensity cannot be considered evidence of any difference.
Conclusions

In multinomial classification, by splitting spectra into up to 36 arbitrary wavelength ranges and building models based on each spectral subrange, we observe improved performance for subranges in the near IR region, where the number of peaks is limited.

The use of model’s VIP and other feature selection methods allow us to reduce the model complexity by removing redundancy and limiting overfitting. Also, the area which contains more VIP>1 shows better model performance. We learn via regularization algorithms that there are important features available beyond wavelength interval ~ 900 and samples>2500 that have the greatest contribution into group’s classification. Eliminating the spectra beyond these ranges causes the reverse effect on the model and is therefore undesirable.

As further work, the intention is to develop a device that is portable, works without a vacuum, and can be used to study argon present in breath in order to identify the presence of disease.
27. MEMS based device concept for future IMS instrumentation in breath research

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Background
An easy and verified detection of biomarkers is the key to access new applications in medical diagnosis and therapy. An interesting group of substances is volatile organic compounds (VOCs), because they can be obtained non-invasive in breath, urine or saliva. In recent years, an increasing number of studies analyzing VOCs as disease biomarkers emerged. Low detection limits in the ppm to sub-ppb range and a large number of different analytes set high demands on the gas sensing instrumentation. For a broad application range, they need to be portable and inexpensive. Therefore, ion mobility spectrometry (IMS) is a promising technique.

Working principles and components of IMS
One main component of an IMS is the ion filter. Common time-of-flight ion filters use the specific drift time that ions need to travel along the drift length. To ensure a sufficient differentiation of analyte ions, the filter needs drift lengths of at least a few centimeters. On the contrary, the FAIMS (field asymmetric IMS) method uses high electrical alternating fields with strengths above 20 kV/cm to separate ions corresponding to their specific ion mobility. The choice of ionization source enables the range of materials which can be ionized. Commercially available UV sources can reach ionization potentials of up to 10.6 eV.

Results
A miniaturized IMS chip with a unique design has been developed. The MEMS device includes the FAIMS ion filter and the ion detector with electrode gaps of a few 10 µm. This new device allows an easy modification of the geometric parameters of the filter and detector. Thus, it can be adapted towards the needs of the specific applications. The poster will focus on: 1) sensor basics and status of development, 2) typical applications and substances, 3) timeline and outlook for ongoing research.

Conclusion
The achieved development level is a promising base for ongoing research and for prospective integration into a demonstration system. This system is expected to be a versatile platform that enables a use in biomedical applications. For this purpose we want to identify collaborators for future projects or research proposals.
28. Human subject study to demonstrate aldehyde levels in breath

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Background

Oxidative stress has been shown to be involved in the development of many disorders, such as ADHD, Parkinson's Disease and vitiligo. As targets of oxidative stress, lipids oxidize into a variety of aldehydes, which exacerbate damage by leading to genetic substitutions and mutations. Thus, researchers have demonstrated that aldehydes can serve as biomarkers for oxidative stress. Current aldehyde tests require a blood draw; we believe breath could serve as a diagnostic tool for oxidative stress levels, but to date, few large-scale clinical trials have been conducted to discover normal concentrations of metabolites in human breath. We sampled nearly seven hundred participants to quantify the distribution and abundance of aldehydes in human breath. This will help us determine what is "normal" for the distribution.

Methods

Participants complete a brief demographic survey, then fill a 10 L Tedlar bag with breath. Metabolites are extracted using solid-phase extraction (SPE) cartridges. A derivatization agent is added to react with aldehydes for improved detection. Samples are analyzed using liquid chromatography-mass spectrometry (LC-MS).

Results

We first conducted an initial confounders study to standardize breath collection. This screening looked at ways that aldehyde levels may be artificially influenced by common activities. With a standardized protocol, our researchers collected samples from nearly 700 participants and have quantified the distribution of breath aldehydes in breath. We have also examined connections between breath aldehyde levels and demographic factors, such as age, gender and ethnicity.

Conclusions

We present our findings of the concentration distribution of breath aldehydes in our sampled human population and findings of correlations to demographic factors.
29. Effect of ingestion of fat burner supplement on breath acetone in exercise experiment

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Background

Acetone contained in exhaled air is expected to be an easy noninvasive indicator of lipolysis in our body, because acetone is one of the final products in lipolysis. Many fat burner supplements to promote lipolysis have recently appeared on the market. We carried out exercise experiments to demonstrate the effect of fat burner supplement taking before the exercise on breath acetone change during and after the exercise.

Methods

All subjects were healthy men (n=5) aged from 22 to 43 years. A bicycle ergometer was used for the exercise. Exercise intensity and duration for each subject were 40% determined by heart rate and 90 minutes, respectively. Breath air was sampled every 15 minutes from 30 minutes before the exercise to 240 minutes after the exercise using a collection bag, and it was analyzed using a FID gas chromatograph. In addition, breath air was also sampled five times in the experiment for one minute using a Douglas bag to measure respiratory quotient and to estimate energy source balance between fat and sugar. All subjects took breakfast (400 kcal) 90 minutes before the exercise. The experiment was carried out two times for each subject on separate days: Subjects took fat burner supplement 30 minutes before the exercise in one experiment and took nothing in the other experiment to compare the effect of ingestion of fat burner supplement on breath acetone.

Results

Breath acetone did not increase during the exercise then significantly increased after the exercise in both, taking fat burner supplement and taking nothing, cases. The increase after the exercise in the case of taking fat burner supplement, however, was lower than that in the case of taking nothing. On the other hand, respiratory quotient decreased after the exercise in both cases, and there was no significant difference in the decrease between both cases. Contrary to our expectation, the increase of breath acetone after exercise in the case of taking fat burner supplement was not higher but lower than that in the case of taking nothing. The supplement provided to the subjects contained glucogenic amino acids such as ornithine and L-carnitine. These glucogenic amino acids might suppress the production of ketone bodies including acetone like sugar. On the other hand, there was no significant difference in the respiratory quotient decrease after the exercise between both cases. The sugar produced from the glucogenic amino acids might be not enough to increase respiratory quotient in the case of taking fat burner supplement.

Conclusions

Breath acetone production was not enhanced by taking fat burner supplement. We should consider the effect of supplements if breath acetone is used as an indicator of lipolysis.
Background

As the number of publications on breath volatiles has grown so has the heterogeneity of sampling and analytical methods. The importance of reproducibility and standardisation of analysis and reporting has led to the formation of the multinational Peppermint Consortium; a group aiming to define benchmarking workflows that can facilitate data comparison between research teams. Here we present the first gas chromatography-mass spectrometry (GC-MS) results from three member groups. We aim to set preliminary benchmark values for the limits of detection (LODs) for breath sample analysis by GC-MS.

Methods

Headspace analysis of off-the-shelf peppermint oil capsules was performed to determine compounds of interest. Ten healthy participants were recruited at each of the three centres. The standard Peppermint protocol was followed: in brief, each participant provided a baseline breath sample prior to taking a peppermint capsule. Samples were collected at 60, 90, 165, 285 and 360 min following ingestion. Sampling and analytical protocols were different for each institution, in line with their usual practice. All samples were analysed by GC-MS and washout curves calculated for volatiles identified in the capsule headspace. LODs were determined based on the intensity of each compound in the background and a linear regression performed to determine at what time point the LODs were breached.

Results

Menthol, menthone, eucalyptol, α-pinene, β-pinene were identified in the capsule headspace and selected as target compounds. Washout profiles were determined for each compound and three general patterns observed amongst participants - rapid, intermediate and slow washout. Preliminary benchmark values for breath samples analysed by GC-MS are presented for each compound and washout group.

Conclusions

We present initial GC-MS findings from the Peppermint Consortium and suggest a benchmark value against which we suggest the analytical performance of future publications on breath volatiles may be compared. We are optimistic that as more groups
join the Consortium to share their knowledge and data we will be able to both improve the quality of our benchmark and reduce the LOD further.
31. On-line analysis of exhaled breath with SESI vs. PTR high-resolution mass spectrometry

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Background
On-line analysis of exhaled breath with PTR and SIFT mass spectrometry are established methods while SESI mass spectrometry is a more recent technology without established quantification. Several novel putative breath markers have recently been reported with SESI-MS, particularly for the high mass region (> 200 m/z). Very recently PTR was coupled to mass spectrometers with high-resolution capabilities (HRMS) comparable to SESI-TOF analyzers used in the clinics. We used this opportunity to compare the simultaneous analysis of exhaled breath with PTR vs. SESI ionization.

Methods
An integrated PTR-TOF mass spectrometer (Vocus PTR-TOF) was connected to the same sample inlet as a SESI ion source (Super SESI) connected to a QTOF mass spectrometer (TripleTOF 5600+). In total 22 healthy subjects were measured with the same exhalations recorded on both systems. In addition, 95 standards from 12 important compound classes (e.g. aldehydes, ketones or fatty acids) were measured with an evaporator system to investigate the formation of different ionization species.

Results
Significant more m/z features were detected with SESI compared to PTR (1702 vs. 1009 m/z features). For the higher mass region (m/z 200 - 500) 920 were unique for SESI. The overlap of m/z values was lower than expected (37 % for m/z 50 - 200) even taking into account common adducts and generic losses. Sensitivity was comparable for both methods while the softness* of the two methods was quite different with different formed ion species (adducts, clusters and losses). This has respective consequences for the separation power and capabilities for unknown identification for with PTR vs. SESI-HRMS. The simultaneous measurement of each exhalation with both methods allowed to determine the detected concentration ranges with SESI-MS for compounds with established PTR-MS quantification.

Conclusions
On-line analysis of exhaled breath with PTR vs. SESI-HRMS resulted in significantly different detected m/z features with a clear mass dependency, different formation of ion species and good correlations for selected breath markers between both methods.
32. Breath analysis by GC×GC-FID/qMS and the search for exhaled biomarkers of acute breathlessness

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Background
The East Midlands Breathomics Pathology Node (EMBER, Leicestershire UK) focuses on the development of analytical technologies for the discovery of novel breath biomarkers to stratify patients with acute breathlessness (i.e. asthma, COPD, heart failure and pneumonia).

Methods
This work describes the development of a method for the analysis of exhaled breath VOCs by comprehensive two-dimensional gas chromatography (GC×GC) with dual FID and MS detection.

Results
Optimisation of the chromatographic separation and efficacy of the method, within a large-scale multi-site clinical study resulted in the successful integration of the method and sampling protocol in-clinic, demonstrated through the collection and analysis of breath samples from over 550 patients.

Conclusions
The results highlight the potential application of breath analysis for advancing precision medicine in acute respiratory diseases.
33. A preliminary investigation into the impact of tonsilloliths on oral malodour using Selected Ion Flow Tube Mass Spectrometry

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Background

Tonsilloliths, commonly known as tonsil stones, are concretions that accumulate in the tonsillar crypts. Tonsilloliths can be classed as microbial biofilms and have been indicated as a potential cause of oral malodour. In some case studies patients have reported both extreme halitosis and discomfort from tonsilloliths.

This study will investigate the extent that tonsilloliths contribute to the oral malodour profile of patients. These analyses will be made using the real-time gas phase sampling technique Selected Ion Flow Tube Mass Spectrometry (SIFT-MS).

Methods

The oral cavity of patients, who sometimes exhibit tonsilloliths, will be sampled daily using SIFT-MS. A visual inspection of the oral cavity will take place to determine if any tonsilloliths are present. The chemical profiles will be compared to determine if a significant difference is observed when tonsilloliths are visibly present.

Additionally, the extracted tonsillolith will be measured away from the oral cavity and SIFT-MS analysis will determine which specific VOCs are evolved.

Results

A preliminary investigation has allowed for the direct VOC measurement of a tonsillolith using SIFT-MS. A two tailed two sample T test has indicated that compounds such as dimethylsulphide, propanoic acid and butanoic acid were evolved at concentrations significantly higher than the control measurements. Whereas, other oral malodour related VOCs, such as trimethylamine and putrecine, were not detected.

Conclusions

The successful real time measurement of VOCs from an extracted tonsilloliths has been successfully demonstrated using SIFT-MS. This preliminary investigation demonstrates that the method holds promise for the potential discrimination of VOC profiles from patients exhibiting tonsilloliths. If successful, these studies could assist with the quantification of tonsillolith contribution to oral malodour.
34. GC-IMS for the Peppermint Consortium: A benchmarking protocol for
breath sampling and analysis.

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Background

Many different methods have been developed to sample and analyse breath, and the
number of publications has grown, there is still little known about the differences between
them. A need for introduction of standardisation for sampling and analysis has led to the
formation of the multinational Peppermint Consortium; a group aiming to define
an experiment to promote data comparison between research teams and studies. Here we
present the gas chromatography-ion mobility spectrometry (GC-IMS) results collected by
two member groups. We aim to present a model with preliminary benchmark values for
the limits of detection (LODs) and sampling precision for breath analysis by GC-IMS.

Methods

Ten healthy participants were recruited by two consortium members. The sampling design
followed the benchmarking Peppermint protocol. Each participant provided 6 breath
samples one before peppermint capsule ingestion and then at 60, 90, 165, 285 and 360
min post ingestion. Sampling and analytical protocols were in line with each institution's
usual practice. A total 25 peppermint data sets were produced and an additional 5 data
sets were provided from outside of the consortium and these were also evaluated. All
samples were analysed using GC-IMS. The identities of peppermint oil volatiles were
identified by headspace injections of pure standards. Calibration and LOD were
determined based on the eucalyptol peak volume and a linear regression performed to
determine at what time point the alveoli gradient was equal to 1 (change in breath
concentration no longer seen).

Results

Six different features were found in breath of participants who had ingested a peppermint
capsule. Eucalyptol, α-pinene, β-pinene were identified using headspace standards and
eucalyptol was the largest feature. Eucalyptol washout profiles were placed into one of
three categories based on the time stamp of the sample with the highest observed
maximum concentration, 60, 90 or 165 min post ingestion. Preliminary benchmark values
for breath samples analysed by GC-IMS resulted in a eucalyptol washout regression model
with $R^2$ values of $> 0.99$. A minimum value for a washout time with Eucalyptol alveoli
gradient $> 1$, was estimated to be no lower than 328 min; the lower 95% confidence limit
of the washout profile. The benchmarking protocol also identified rapidly experiments,
protocols and methods that were not producing reliable and reproducible data.
Confounding factors (age, BMI, diet) were identified as influencing eucalyptol washout
profiles.

Conclusions

The first benchmark findings for GC-IMS from the Peppermint Consortium are presented
and a benchmark value of 328 min is proposed for the minimum time from ingestion of a
peppermint oil capsule that eucalyptol may be detected with an alveoli gradient $>1$. We suggest a format of peppermint washout results for supporting future reports of GC-IMS studies on breath.
Background
Antimicrobial resistance is set to be an unprecedented threat to modern medicine. 'Sniffing' bacteria potentially offers a rapid way to determine susceptibility.

Methods
Thermal desorption-gas chromatography-mass spectrometry was used to 'smell' cephalaxin and ciprofloxacin resistant and sensitive urinary tract infection-causing bacteria in culture.

Results
578 peaks at unique retention times were identified from 86 chromatograms of 18 bacterial isolates (E. coli, K. pneumoniae and P. aeruginosa). The isolates were grown with and without the presence of antibiotic. Chi-square analysis found 9 retention times that differed significantly between cephalaxin sensitive and resistant isolates, and 22 retention times that differed significantly between ciprofloxacin sensitive and resistant isolates, at p = <0.05. When antibiotic was added to the media, more differences were found in the cephalaxin group, attributed to lysis, but not in the ciprofloxacin group.

Conclusions
A proof-of-principle study has been completed. Further work with larger sample sizes will validate these findings and potentially enable the development of diagnostic algorithms using presence/absence of particular compounds of interest.
36. Exhaled chemicals associated with molecular hydrogen via colonic fermentation after the ingestion of soybean

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Background:
Soybean contains considerable amounts of dietary fibers. Ingestion of soybean markedly increases breath hydrogen molecules (H2) via anaerobic colonic fermentation. Reportedly, there are nearly 400 kinds of volatile compounds released by anaerobic fermentation in healthy human. In the present study, we examined how ingestion of soybean affects components of exhaled air via the colonic fermentation by the comparative analysis of exhaled chemicals associated with colonic H2 fermentation.

Methods:
Healthy adult volunteers participated in the study. After starvation of 12 hours, breath H2 was analyzed every 1 h for 9 h by gas chromatography with a semiconductor detector and another volatile compounds by ion mobility spectrometry.

Results:
In exhaled air, we identified 26 and 27 kinds of volatile compounds that indicated significantly positive and negative correlations to exhaled H2, respectively.

Conclusions:
All of these chemicals reported to exist both in bacterial fermentation and exhaled air. We considered that conditions of H2-associated colonic fermentation could be detected by the analysis of various kinds of exhaled air.
Clinical application of negative photoionization IMS: Point of care monitor of blood propofol concentration during total intravenous anesthesia

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Background

The potency of intravenous agents is expressed in terms of the blood concentration. Due to the complexity of monitoring technology, there are no appropriate methods to measure blood propofol concentration in real-time during anesthesia. Our group constructed a negative photoionization IMS (NP-IMS) to measure the plasma propofol concentrations without any sample pre-treatment (Wang et al., Scientific Reports, 2016). This study aims to verify the feasibility of this instrument in clinical.

Methods

The study included 9 patients of American Society of Anesthesiologists physical status II or III, scheduled to undergo elective surgery. Anesthesia was managed by a target controlled infusion (TCI) device (Orchestra Base Primea, Fresenius Kabi, France). Propofol (Diprivan; Astra-Zeneca, Caponago, Italy) and remifentanil were infused to achieve target plasma concentrations of 5μg/ml (Marsh model) and 5 ng/ml (Minto model), respectively. After intubation, propofol was maintained at target plasma concentrations of 2.5~3.0μg/ml, several blood samples and plasma samples centrifuged from blood were obtained and simultaneously analyzed by NP-IMS (blood, plasma) and HPLC (plasma). Relationships of blood concentration measured by NP-IMS (blood, plasma) and HPLC (plasma). Plasma concentration measured by NP-IMS (CP-IMS) and plasma concentration measured by HPLC (CP-HPLC) were investigated.

Results

The drift time of propofol was constant at 7.28ms in all of the measurement. The repeatability was found to be satisfactory with relative standard deviation (RSD) of 4.5% and 3.9% for 5 times measurement of a 5μg/ml blood/plasma propofol concentration. Good accordance was showed in CB-IMS, CP-IMS, CP-HPLC, and propofol plasma concentration predicted by TCI at different time points. Linear relationships were found in 166 pairs of CB-IMS and CP-HPLC samples (r²=0.927), and 163 pairs of CP-IMS and CP-HPLC (r²=0.925).

Conclusions

The optimal relationship between CB-IMS and CP-HPLC suggested NP-IMS may offer a convincing method to be a point of care detection in blood propofol concentration monitor in clinic. One drop of blood within one minute analysis provides a rapid feedback of propofol concentration to the anesthetists, which may be important in developing of closed-loop anesthesia.
Use of cellular ethanol metabolism for screening the effects of volatile agents

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Background
Many agents with insufficient toxicity data are gas phase and cannot be examined using standard cell culture, with media impeding the delivery of the agent to cells. We are developing a system using cells that can tolerate a lack of fluid medium on the apical side for potential screening of gaseous agents. Changes in the conversion of an exogenously added substrate to a gaseous metabolite in vitro can be utilized to monitor and screen biological responses induced by an agent of interest (i.e., “probe molecule approach” to toxicity testing).

Methods
BEAS-2B human airway epithelial cell line cultures were incubated with or without ethanol (1-2 %) in a flow-through system and the production of acetaldehyde (C2) and other gaseous carboxyls were collected on the outflow line with dinitrophenylhydrazine packed cartridges. Samples were analyzed by HPLC-UV.

Results
Results showed increased amounts of C2 with ethanol exposure (1 and 2% for 2 and 6 hr, respectively) compared to vehicle controls. Control blanks showed negligible C2 background. Cell viability was >95% by trypan blue exclusion. In separate studies where the cells were allowed to desiccate, carboxyls in the C4-C6 range were observed, suggesting these carboxyls are possible gaseous biomarkers of cell death.

Conclusions
The use of BEAS-2B cells and this flow-through gas system shows promise for 1) being able to expose cells to a volatile substance (ethanol) and preserve cell viability for several hours, and 2) capture a gas phase metabolite for use in probe molecule approaches for screening the induction of toxicity by a gas phase agent. Further optimization of the system is underway. The use of in vitro metabolism for chemical screening may reflect in vivo metabolism responses that can be captured in exhaled breath. [This abstract may not reflect official US EPA policy.]
39. Investigating the relationship between skin volatile acid emissions and surface pH

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Background

The outer surface of skin, the stratum corneum (SC), also known as the acid mantle, has an acidic pH which is important for controlling the resident microflora on the skin as well as supporting important physiological processes such as maintaining an optimal structure of the lipid barrier and SC homeostasis.

Maintaining the skin’s pH within the normal range (4.5-5.5) is important for healthy skin. Any fluctuation can trigger dryness, itchiness and other forms of dermatitis. Typically, skin pH is measured using a pH probe and more recently with wearable colorimetric sensors which require direct contact with the skin for extraction and analysis of small volumes of sweat. Our group are investigating a new method of assessing skin pH via volatile acids as an alternative to the present approaches which rely on measuring the non-volatile acid content from a liquid matrix (e.g., sweat). Using volatile acids as a means to assess pH could reduce sample handling steps by eliminating the need to extract fluid from the pores of the skin, as the volatile acids are being released in gaseous form spontaneously from the skin.

Methods

We have characterised volatile acid emissions from skin in healthy participants using a wearable headspace solid phase microextraction (SPME) approach with gas chromatography-mass spectrometry (GC-MS) in addition to colourimetric sensor arrays comprising of pH sensitive dyes. The relation between the volatile acid component and skin surface pH is currently under investigation.
40. Investigating volatile emissions from skin as prospective markers of barrier function

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Background

Human skin is a region of high metabolic activity where secretion of a rich variety of biomarkers occurs throughout the stratum corneum. The skin is a constant source of volatile organic compounds (VOCs) derived from the skin glands and the resident microbiota. Skin-derived VOCs contain the footprints of cellular activities and are receiving significant interest as prospective diagnostic markers of cutaneous disease.

Methods

A small wearable concentration platform integrating solid phase micro-extraction (SPME) was developed for localised skin volatile sampling. Samples from 10 healthy participants (n=60) were collected and analysed with gas chromatography-mass spectrometry (GC-MS) towards understanding volatile profiles before and after acute barrier disruption.

Results

Discriminating volatile profiles were observed for all participants before and after barrier disruption. Volatile emissions revealed the protective hydrolipid film that function within the skin barrier was impacted, with fatty acid and squalene degradation products showing substantial changes after barrier disruption.

Conclusions

This approach could enrich understanding of skin barrier function and treatment efficacy which are key to improving outcomes for chronic conditions like atopic dermatitis and psoriasis. A clinical study is currently underway to investigate volatile emissions these prevalent skin barrier diseases.
41. Automated analysis of raw gas chromatography-mass spectrometry breath samples by a deep learning based system

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Background

Human breath carries thousands of volatile organic compounds (VOCs), measurable with gas chromatography-mass spectrometry (GC-MS), which comprehensively describe the individual's health conditions. Breath analysis has the potential to deliver a fast, accurate and non-invasive diagnostic platform. However, detection of the VOCs in GC-MS data requires time-consuming expert-driven processing, which is prone to errors and delivers operator-subjective results.

Methods

We propose a system, employing deep learning -- precisely convolutional neural networks (CNNs), to learn and automatically detect VOCs' patterns directly from raw GC-MS data, bypassing expert-led processing. We evaluate this CNN-based approach on clinical samples and with four types of networks: VGG16, VGG-like, Densely-connected and Residual CNNs.

Results

All system configurations not only demonstrated high sensitivity and specificity but also detected approximately 25% more VOC occurrences than expert-led analysis.

Conclusions

Our results indicate that the automated CNN-based method can improve accuracy and reduce the time of samples analysis, vitally contributing to the large-scale development of breath-based diagnosis. The system may support experts to put much more accurate hypothesis on the VOCs related to the specific health conditions. Moreover, by the significant acceleration of the VOC detection process, the CNN-based method allows for much quicker hypotheses validation on new GC-MS samples.
Background
Commensal Candida spp. within the lung environment rarely cause harm to healthy individuals, however their invasive forms are a major concern for hospitalised patients with weakened immune systems, commonly resulting in severe illness. Candida spp. are highly adaptable to their surrounding environments due to their dimorphic capability and ability to form synergistic relationships with other commensal lung microbes such as Staphylococcus aureus. Microbial-derived volatile organic compounds have shown promise as markers of lower respiratory tract infections (LRTIs) through breath analysis, however changes in environmental conditions surrounding infection and how these may impact the VOC profile are not well characterised. The aim of this work is to investigate changes in the VOC profile of C. albicans under different environments and in co-culture with other LRTI pathogens.

Methods
A sampling method was developed which involved actively sampling headspace gas onto thermal desorption tubes from within headspace vials containing microbial cultures. In this experiment, we cultured C. albicans ATCC10231 in two growth media, Artificial Sputum Media (ASM) and Sabouraud Dextrose Broth (SDB), and sampled the headspace above cultures. Cell concentrations were standardised before being incubated for 24h at 37 °C. After incubation, 100 mL of headspace gas was sampled onto Tenax TA sorbent tubes, replaced with filtered air. Tubes were then analysed by Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS). Peaks were integrated using Masslynx software, and VOCs identified through the NIST mass spectral library. Relative standard deviation (RSD) were calculated from five replicates.

Results
An average of 41 and 38 chromatographic peaks were successfully integrated for C. albicans cultures in ASM and SDB, respectively. Six were associated with C. albicans cultured in ASM (RSD 15.2-37.1%), namely heptane, 3-methyl-1-butanol, 2-methyl-1-butanol, n-hexyl methanoate, 3-octanone, and 2-octanone. Eleven microbe-derived VOCs were identified in SDB cultures (RSD 6.5-79.8%), including 2-methyl-2-propanol, ethyl acetate, 2-methyl-1-propanol, ethyl propanoate, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl butanoate, Isoamyl acetate, and three unidentified sesquiterpenes. Both 3-methyl-1-butanol and 2-methyl-1-butanol were identified across both ASM and SDB C. albicans cultures.

Conclusions
We have demonstrated that changes in the culture environment can influence the VOC profile for C. albicans. Future work will involve optimisation of the headspace sampling method to analyse the headspace of co-cultures and changes in environmental conditions which may impact the VOC profile as well as microbial virulence.
Background

Invasive aspergillosis (IA) is a major fungal infection, which can cause severe illness and mortality to hospitalised patients with a weakened immune response. Triazoles are the first-line treatment option for patients diagnosed with IA, however resistance to compounds in this class are emerging. In this study, we explore the potential of volatile metabolites from culture headspace to distinguish between azole-resistant A. fumigatus TR34/L98H from a laboratory-type control.

Methods

Conidiospores were harvested, filtered, and standardised before incubating for 48 h at 37 °C on Aspergillus minimal media. Headspace from pure cultures was then sampled onto thermal desorption tubes at 100 mL min⁻¹ for 2 min. TD tubes were then analysed by Thermal Desorption-Gas Chromatography-time of flight-Mass Spectrometry. Untargeted analysis was performed and extracted features were identified using the NIST (v.14) library.

Results

On average, 123 VOCs were detected in the headspace of isogenic TR34/L98H (n=11) and 125 VOCs for WT controls (n=8). Known VOCs putatively associated with A. fumigatus were found in both WT and TR34/L98H, including ethyl acetate, pyrazine, methylpyrazine, α-pinene, camphene, limonene, himachalene, and α-bergamotene. Six VOCs showed significant changes in abundance between the control and TR34/L98H strains (p < 0.02), one of which originated from the media.

Conclusions

In this study, we demonstrate that fungal volatile metabolites may be used as markers of azole-resistance in-vitro, with potential applications in exhaled breath volatile analysis.
The impact of food consumption on sensor readings in a POC breath analysis device

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Background

Breath testing systems have proven to be valuable and accurate tools in laboratory settings, but a point-of-care breath analysis system can be used for various disease detection tests in an online mode (testing on-site and receiving the result immediately after). However, it is sensitive to everyday sources of VOCs like environmental factors, food and drink consumption before the test and hygiene routines of the tested person. This study tests for differences in sensor response after a person consumes a standardized meal.

Methods

A group of people (15 individuals) were recruited for an experimental study using a point-of-care modular breath analyser prototype, which included three modules with different type of sensors: 8 gold nanoparticle sensors (GNP) in development stage, 8 commercial analogue and 10 commercial digital metal oxide (MOX) sensors. The first test was carried out on breaths after 12 hours of fasting, then the participants were given a standardized meal and invited for a follow-up test 4 hours later to test for differences in the pattern of the breath analysis sensors. The readings of each sensor were analysed.

Results

When testing to see if all sensors ‘recognized’ breath (statistical tests between mean baseline (room) air readings and mean breath readings), 96.2% of the sensors showed significant difference (p<0.05). When testing for difference in breath before and after food consumption, 12.5 % of GNP sensors, 87.5% of analogue MOX sensors and 70.0% of digital MOX sensors showed statistically significant differences.

Conclusions

The sensitivity to food consumption was dependent on the type of sensors. Most of the GNP sensors were not sensitive to food consumption. Most of the analogue MOX sensors discriminated well between breath before and after food consumption. Seven of the sensors in the digital MOX module showed statistically significant differences in readings of breath before and after food consumption. This leads to a conclusion that, although not all sensor readings seem to be affected by food consumption, it should be considered as a potential interference and taken into account when designing a proper sampling protocol.

Funding
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Improved discovery of biomarkers of disease by TD-GC×GC-TOF MS

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(2) Markes International, Llantrisant, UK

Background
The accurate identification and measurement of biomarkers in biological samples - such as breath, saliva and urine - has the potential to provide rapid, minimally-invasive diagnosis of a range of physiological and pathological conditions, resulting in the delivery of precise medicine.

Pre-concentration techniques - such as solid phase microextraction (SPME), sorptive extraction and thermal desorption (TD) - in combination with secondary focusing on a cold trap provides the required sensitivity. It also has the advantage of optional re-collection of a portion of the sample, either for storage, or to re-analyse it to confirm the compounds identified, which is advantageous when trying to identify biometric patterns.

Analysis by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) then provides the enhanced separation necessary to resolve these complex mixtures. In metabolomics matrices, diagnostic compounds are rarely of high abundance, and by adopting a comprehensive approach, measurement of the maximum possible number of compounds is achieved in a realistic run time.

Methods
Here, we will demonstrate the enhanced performance of this multi-functional TD-GC×GC-TOF MS system by simulating disease biomarkers through a controlled, artificial change in participants’ breath. Breath samples were collected before and after ingestion of a peppermint capsule in an attempt to mimic changes in metabolism. The enhanced separation of the GC×GC system ensures that trace metabolites are not masked and/or overlooked and provides cleaner spectra for confident identification of the pseudo-biomarkers.
46. A roadmap to breath and biofluid analysis based upon thermal desorption

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Background
Clinical studies involving Breath analysis by means of Thermal Desorption (TD)-GCMS are based on a multi-step, multi-locations workflow.

Methods
The sample is first collected in the clinical setting: several solutions are nowadays available for the collection and transfer of exhaled breath onto thermal desorption tubes, but ideally a breath sampling device should be portable and easy to use and made of low absorption, low-emitting materials, thus minimising the introduction of exogenous analytical factors. Following collection, breath analysis is usually centralised within a regional analytical lab: this imposes analytical constraints related to sample transport, storage and security.

The use of thermal desorption provides a solution to these issues because:

1. TD tubes used to collect and store exhaled breath samples are compact in size, so easy to handle and transport, and can be sealed and stored for extended periods of time, thus ensuring sample integrity.

2. Although clinical samples are unique and invaluable, repeated automated analysis is always possible: a breath sample taken in the framework of a clinical study captures a unique snapshot of the patient’s clinical journey and it is often unrepeatable. As a consequence, if for any reason that sample is not correctly analysed, it will be irreparably lost. Thermal desorption offers the possibility to re-collect a representative fraction the original sample, allowing for repeated confirmatory analysis of these unique clinical samples.

3. Thermal desorption – GCMS is highly automated and provides all the tools for necessary QC of the entire workflow, from sample collection to data interpretation, thus enabling high-throughput, high-quality routine analyses.

This poster presents a range of solutions available for the collection and analysis of exhaled breath, followed by GCMS analyses.
47. Thermal Desorption: the optimum way to ensure quality in breath analysis

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Background

The implementation of breath analysis in clinical practice requires to confirm the data acquired on pilot scale employing larger patient cohorts. In large-scale clinical trials, breath analysis is carried out over time spans of several months or sometimes years, involving multiple locations. Due to these challenging conditions, breath researchers worldwide are faced with an analytical dilemma: how to prove with confidence that data are of good quality? Large scale metabolomic studies using other sample types, such as blood or urine, are traditionally more well-established;1 within the context of these studies, several types of samples can be prepared with the aim to assess data quality, these include:

- ‘System suitability samples’ allowing you to check if analytical instrument is ‘fit for purpose’ before wasting invaluable patient samples.
- ‘Internal standards’: reference compounds, added to every sample. Optimal choice is the non-radioactive isotopologue (e.g. 13C or D) of the analyte(s) of choice.
- ‘Intra-study pooled samples’: prepared to assess instrumental variability over time. It can be obtained by mixing small aliquots of real samples, or by preparing an artificial QC sample reproducing the matrix of interest.
- ‘Within laboratory inter-study samples’: these are provided by an external authority and can be used in round robins including different labs.

This approach to standardisation is traditionally less applied in breath research, but if breath analysis enters clinical practice, all these types of standards could be required. This poster shows how Thermal Desorption allows utilising all of them: standards can be liquid or gaseous, they can be prepared in-house or purchased and if necessary obtained from a third-party authority as certified products. Standard addition can be performed manually or in automated fashion. TD also offers the option to add standards to the focusing trap in order to assess GCMS instrumental response over time or to tube, either before or after sample collection, to track the quality entire sampling protocol. In summary, automated TD-GCMS analysis provides breath researchers with the opportunity to make breath samples more amenable to QC checking without using cumbersome workflows.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) has been proposed as a powerful technique for the analysis of complex biological samples.

The commercialisation of consumable-free, modulators have only strengthened its position for the analysis of breath volatiles – where routine analysis of large sample batches is essential. Here, we will demonstrate the performance of a robust and repeatable flow modulator using best-in-class, reverse fill/flush dynamics.

The aim of this study was to develop a robust GC×GC method for the collection and analysis of breath volatiles. Optimisation of the system will be demonstrated - from the sampling of breath onto sorbent tubes for thermal desorption (TD), through to the data analysis workflows - using sorbent tubes spiked with a suite of known biomarker standards. We will show the repeatability and linearity of the system, prior to the analysis of breath samples.

Furthermore, we will show how automation can increase throughput and precision, with automated spiking of the sorbent tubes using gaseous or liquid standards, automated TD with unique tube caps to maintain sample integrity and automated recollection of split flows for repeat analysis.

The results demonstrate a comprehensive method for the collection and analysis of VOCs in breath by TD-GC×GC-TOF MS.
49. Dynamic headspace sampling for optimal extraction of fecal volatile organic compounds using a micro-chamber

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Background

Volatile organic compounds (VOCs) have been demonstrated as potential non-invasive diagnostic biomarkers in various clinical and preclinical studies. VOCs can originate from various (patho)physiological processes occurring in the human body and they can be detected in different body-fluids, including blood, exhaled breath, urine and feces.

VOCs present in the headspace of feces have been exploited as diagnostic biomarkers for many gastrointestinal diseases, such as inflammatory bowel disease, irritable bowel syndrome or celiac diseases. VOCs in fecal headspace are typically sampled using solid phase micro-extraction (SPME) or dynamic headspace extraction. SPME has prominently facilitated the isolation and analysis of VOCs in human feces. However, dynamic headspace sampling offers a robust way of analyzing volatiles in fecal samples.

Methods

In the current study, for the first time, the dynamic headspace of fecal samples has been investigated using a micro-chamber/thermal extractor (Markes International Ltd). The micro-chamber is a versatile and compact unit with up to six small cylindrical chambers that enables the sampling of VOCs released from various materials at defined temperature via their collection onto sorbent tubes.

The main aim of the study is to find the best headspace sampling conditions for fecal analysis of VOCs. For that purpose various conditions are tested, including homogenization procedures of the fecal sample (water versus NaCl), fecal sample mass, equilibrium and extraction time, as well as exposure temperature and purge flow.

Results

Fecal samples are collected and frozen at -20°C on the day prior to analysis. Subsequently, they are thawed at 4°C to minimize the effect of fermentation, and placed in the micro-chamber for VOCs extractions. Combinations of the conditions were investigated in order to explore optimal sampling strategies. The VOCs from fecal headspace are analyzed by gas chromatography coupled to mass spectrometry.

Conclusions

The study will lead to the creation of standard operating procedures (SOPs) that will be further used for detecting fecal biomarkers for colorectal cancer in a current multi-center European study in Maastricht (Netherlands,) Munich (Germany), and Torun (Poland).
50. Multi-centre cross-validation study in the search for volatile colorectal cancer biomarkers in breath and faeces

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Background

Non-invasive diagnostic tools based on the detection of volatile biomarkers in exhaled breath have largely failed to reach the necessary maturity to offer screening for practical use. Despite extensive studies and reports on volatile biomarkers (or biomarker sets or patterns) relating to one disease or another, an absence of reproducibility between independent trials throws doubt on their reliability. Cross-validation of large cohorts between clinical/analytical centres is imperative in developing biomarker-related screening tests with high sensitivity and specificity and low false-positives.

Methods

A current multi-centre European study in Munich (Germany), Maastricht (Netherlands) and Torun (Poland) is exploring the presence of disease-specific volatile organic compounds (VOCs) in exhaled breath and stool headspace of colorectal cancer (CRC) sufferers in comparison to other bowel disorders and healthy controls. In the current first phase of the project, standard operating procedures (SOPs) are being established to ensure optimum sampling and analysis of the two gas matrix types. Exhaled breath will be collected on Tenax/Carbograph adsorption tubes (biomonitory tubes; Markes International, UK) using a ReCIVA device (Owlstone Medical, UK) and stool headspace will be extracted onto similar tubes using a micro-chamber/thermal extractor (µ-CTE; Markes International). VOCs will be analysed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS; different manufacturers).

Results

The study design of these trials is unique in that triplicate samples will be collected for each patient in each cohort (breath and stool headspace), and these replicates will be analysed in each of the analytical centres. The project aims at recruiting approximately 300 participants for each cohort (CRC, irritable bowel syndrome, inflammatory bowel disease - including Crohn's disease and ulcerative colitis - and healthy controls) across the three centres. These cohorts will be used to search for CRC-specific biomarker. The selected sets of VOC biomarkers will be further validated in the independent patient cohorts (including 75 individuals across the three centres). This multi-centre design thereby not only offers the opportunity to examine samples from cohorts at three separate locations, but simultaneously provides independent cross-validation (in triplicate) of all samples gathered. Datasets from each centre will be pooled for subsequent data-mining and for biomarker discovery using sophisticated machine learning techniques.

Conclusions
The clinical phase of the project will commence this autumn once the necessary SOPs have been established and optimised and after proficiency training of clinical personnel for sampling and of laboratory personnel for the analyses. This presentation will outline the cross-validation concept of the trials, the methods being employed, and is intended to spark debate and seek advice before the project enters its clinical phase.
51. Characterising volatile organic compounds (VOC) from colonising pathogens related to cystic fibrosis by headspace secondary electrospray ionisation high-resolution mass spectrometry (SESI-HRMS).

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(4) ETH Zurich, Department of Chemistry and Applied Bioscience, Zurich, Switzerland

Background:
Cystic fibrosis (CF) is the most common metabolic disorder in Western Europe. This autosomal recessive disease results in severely reduced mucus clearance in the lungs. Although normal at birth, the airways of CF patients are rapidly colonised by pathogens, exacerbated by the buildup of thick mucus providing an optimal environment for bacterial growth. VOC’s in breath can reflect pathogen metabolism and could offer a non-invasive procedure for early detection of pulmonary infections.

In this study, we propose to differentiate between 6 pathogens commonly detected in the lungs of CF patients using headspace coupled SESI-HRMS and to identify pathogen-specific markers for later comparison with the breath fingerprint of CF patients.

Methods:
Six pathogens were subcultured and incubated at 37 °C in CO2 for 24 h. Inoculation by these control strains was subsequently done in the same medium for cultivation at 35°C in air for 24 h. A sample was transferred to a headspace vial for measurement by SESI-HRMS. Thirty randomly ordered independent experimental repeats were done per strain.

Results:
Principal component analysis (PCA) was found to successfully discriminate between the 6 pathogen groups. In a supervised approach, linear discriminant analysis (LDA) showed an average accuracy of the pathogen profiles of 99.9%. Utilising an extensive list of published pathogen biomarkers in conjunction with a correlation matrix to assign common adducts, we were able to put together a robust list of markers for each pathogen strain for further compound identification.

Conclusions:
Employing a robust methodology, we were able to successfully differentiate between all 6 pathogens using headspace coupled SESI-HRMS in conjunction with common statistical testing. Comparing these pathogen-specific markers with the breath fingerprint of CF patients will allow us to evaluate the applicability of SESI-HRMS for clinical use in early CF diagnosis.
52. Changes In Volatile Organic Compounds In Exhaled Breath Of Cystic Fibrosis Patients After Start Of Lumacaftor/Ivacaftor (Orkambi) Treatment

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Background: Cystic fibrosis (CF) is characterized by the lack of functional cystic fibrosis transmembrane conductance regulator (CFTR) which causes dysregulation of epithelial fluid transport in the airways and a disease specific microbiome adapted to CF lungs. Novel CF therapies such as Lumacaftor/Ivacaftor (Orkambi) target the CFTR and increase its activity. Restoring of CFTR function will change the epithelial fluid transport in the airways of CF patients. Consequently, changes in the microbiome can exist. In this study, we used exhaled breath metabolomics as a non-invasive surrogate marker for host- and bacterial metabolism. We aimed to identify changes in exhaled breath volatile organic compounds (VOCs) before and after start with Lumacaftor/Ivacaftor (L/I) therapy.

Methods: This was a single-center longitudinal observational study in homozygous Phe508del adult CF patients who started L/I therapy. Exhaled breath was collected before and every three months after starting L/I, up to one year. We identified VOCs using Gas Chromatography – Mass Spectrometry (GC-MS). Each VOC was compared in a paired sample T-test between the visit before start of L/I and the first and last visit after start. P-values were corrected for multiple testing. The percentage of VOCs significantly different before and after treatment was calculated.

Results: We recruited 20 patients and all started with L/I treatment. Breath samples of 13 patients were available 1 year after starting treatment. Exploratory analysis showed that 173 VOCs (75.9%) changed in concentration 3 months after start of L/I treatment. The majority (94.8%) of these VOCs remained significantly different at 12 months.

Conclusion: Lumacaftor/Ivacaftor treatment changes the VOCs in exhaled breath of CF patients, both after 3 as well as after 12 months of therapy.
Background: Crohn’s Disease (CD) is a form of Inflammatory Bowel Disease that affects any part of the gastrointestinal tract, from the mouth to the anus. CD may lead to debilitating or even life-threatening complications, and it usually requires heavy medication. CD patients undergo periods of non-active and active stages of the disease. Symptoms during the active phase of the disease include severe abdominal pain, bloody diarrhoea, as well as high fever. This inflammatory stage of the disease is highly related to changes in human metabolome, volatilome, and the gut microbiome. Affected and inflamed organs in CD patients produce, and therefore, release Volatile Organic Compounds (VOCs) in blood and exhaled breath, as well as specific metabolites in plasma. At the same time, gut microbiome dysbiosis is also observed. Previously, analyses of VOCs in breath and gut microbiome, individually, have successfully differentiated CD patients in the active stage of the disease from remission with prediction accuracies of 80% and 82%, respectively. The present study aims to fuse data from four -omics platforms (i.e. VOCs in breath, VOCs in blood headspace, metabolites in blood, and microbial Operational Taxonomic Units (OTUs) in the gut) to enhance the prediction accuracy of the disease activity in CD patients.

Methods: In the present study, 130 breath, faecal, and blood samples were collected from CD patients while visiting an outpatient clinic, and they were classified into active (n = 64) and in remission (n = 66) cases by using a combination of biochemical biomarkers and the Harvey Bradshaw index. VOCs in breath were measured by Gas Chromatography time-of-flight Mass Spectrometry (GC-tof-MS), while VOCs in blood headspace were measured by GC/GC-tof-MS. Metabolites in blood were analysed by Nuclear Magnetic Resonance, whereas OTUs in faeces were assessed by 16S rRNA pyrosequencing. To fuse the data, several data fusion strategies were applied, compared, and evaluated: mid-level, high-level, as well as Multiple Kernel Learning (MKL), a specific case of fusion approach.

Results: Random Forest (RF) and Gradient Boosting Trees (GBT) succeeded in finding discriminatory features to be concatenated in the mid-level fusion case and to get predictions to be fused in the high-level fusion case. RF and GBT were also implemented in the MKL fusion attempt. All fusion strategies were evaluated based on their sensitivity and specificity to detect disease activity in CD patients.

Conclusions: The fusion strategies, as mentioned above, demonstrated a comparable or improved prediction accuracy, and at the same time, correlations among all these data platform variables were found.
54. Effect of inhaled acetone concentrations on exhaled breath acetone concentrations

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Background:
When measuring breath concentrations of volatile organic compounds (VOC) many factors can have an influence on data. For VOCs with high blood:air partition coefficients such as acetone or ethanol the breath concentration equals the concentration in the upper air ways whereas for VOCs with low blood:air partition coefficients such as isoprene or butane the breath concentration equals the alveolar concentration. Also an increase of blood flow or hyperventilation will change breath concentrations. Mathematical models help to understand this influencing factors better.

In a recent paper [Ager 2018] we created a simple three compartment model which theoretically describes the influence of inhaled concentrations on exhaled breath concentrations for volatile organic compounds with higher blood:air partition coefficients.

Methods:
Here we released different acetone and deuterated acetone concentration levels into the laboratory 's air. Due to the building's ventilation system the concentration levels decreased then slowly. Before, during, and after the release we measured continuously the inhaled and exhaled acetone and deuterated acetone concentrations of 3 volunteers through a mask in 7 sessions. The duration of each session was approximately 40 minutes with 10 minutes pedaling on a supine ergometer. For our analysis we used a PTR-TOF-MS with a sampling rate of 5 Hz.

Results:
As predicted the exhaled acetone concentration is a linear function of the inhaled acetone concentration. This observation was already made by [Spanel 2013]. Exercise raises the level of normal exhaled acetone concentration and decreases the level of deuterated exhaled acetone concentration which is exactly what one would expect from our model.

Conclusions:
Ambient air concentration of acetone contribute to the exhaled concentration and therefore approximately 50% of the inhaled acetone concentration must be subtracted for correction.


Monitoring of physiological effects of Winter Swimming by FeNO

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Background

Winter swim is a widely proved intervention training in Rehabilitation. Positive reports are known about the long-term effects on conditioning and stabilization of the cardiovascular and respiratory regulation. Objective biomarkers for monitoring the effects are not yet been proven.

Methods

In the study, 21 subjects, including 10 with known asthmatic disease but complain-free and with unremarkable lung function, were included. Measurement of the FeNO was carried out with a pre-series device of the BC-FeNO (BecherConsult). The device is in compliance with ATS/ERS Guidelines for FeNO measurement. FeNO was measured 2 h before and 2 h after a one-off exposure to cold water for up to 2-3 minutes.

Results

Functional limitations of the respiratory function have not been registered. The mean of the FeNO in asthmatics was on average 34.2 before intervention, (22.6 in lung healthy) with p<0.005 and 26.4 (resp. 21.8) ppb NO 2-3 hours after the Intervention at rest (p<0.05).

Conclusions

In asthmatics, the FeNO value fell by about 33% after a one-off intervention with winter swimming, while in lung healthy there is no change. Whereas the FeNO is an inflammatory marker of the respiratory tract, the intervention seems to have an immediate positive impact on airway inflammation in the short term.

Long-term effects should be the aim of further research.

This study was once again a reference to physiological effects of winter-Swimming.
Background
The possibility to prove infections by spectral analyses from exhaled air and Headspace of bacterial cultures in vitro is already proved. The question remains if markers are released by the bacteria or by the Host or from Host-Bacteria interaction. The study was aimed to check whether IMS is suitable as a screening breath test for infections.

Methods
Patients in an emergency room performed a single breath test and were subjected to a test for air-suction from nose-cavity. The VOC-dependent peaks in IMS-spectra were determined and compared by means of cluster analysis-based software. The classification has been carried out with a leave-one-out cross validation and support vector machine.

Results
In calculation of nasal and bronchial samples of MRSA (15), VRE (10) and E. Coli (12) positive classification were included. Significant clusters due to the pregrade classification were found, indicating the germ, and furthermore for determination of the sample, e.g. nasal or peroral. Pre-determined selective clusters for differentiation of a specific germ were found regardless of the origin of sample.

Conclusions
Using different samples for determination of a germ some specific markers will be found but also more different markers giving a hint for the origin of the sample. Nasal sample suction seems to be more easier and reproducible than peroral sampling in routine application.
57. Non-invasive monitoring of blood sugar levels by saccharoids in Exhaled Breath Condensate (EBC) - the 2nd report

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Background: Diabetes Mellitus (DM) is one of the leading causes of death and disability in these days. As DM control relies on patient adherence to routine blood glucose level (BS) monitoring, noninvasive method of the monitoring, such as breath test, is eagerly desired. We previously reported detection of trace glucose and lactose in exhaled breath condensate (EBC) of healthy subject using thermal preconcentration and LCMSMS. The purpose of the study is to compare the time course of EBC sugar levels after sucrose ingestion with that of BS, and verify whether EBC sugar analysis can estimate real-time changes of BS.

METHODS: 12 healthy subjects received 100 g of sucrose after a 12hrs fasting. EBC were collected before and 30, 60, 120 minutes after the ingestion. Capillary BS were measured simultaneously. EBC glucose and lactose levels were measured using LCMSMS with multiple reaction monitoring.

RESULTS: There was no significant correlation between BS and EBC glucose or lactose levels. However, there was a significant correlation (p <0.05) between BS and EBC glucose / lactose ratio. Linear model analysis gave BS(mg/dL) = 302.7 × EBC glucose-lactose ratio + 72.1, Spearman's rho (95% CI) = 0.70 (0.49-0.91). BS peaked at 30 minutes after the ingestion, then gradually declined to recover the baseline at 120 minutes. The time course of the EBC glucose-lactose ratio resembled that of BS.

CONCLUSION: The results showed possibility and validity of the EBC glucose-lactose ratio as an indicator of real-time BS fluctuation. This study provides still a small but important step towards totally noninvasive BS monitoring by breath test.
Bioequivalence test for generic drugs using 13C breath test as a non-invasive replacement of blood test

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Background: Prior to the marketing of generic drugs (inexpensive alternative drugs), bioequivalence test (BET) is applied to confirm their absorption and metabolism are comparable to the original drug by pharmacokinetic analysis. According to the Japanese BET guidelines, the analysis requires at least 7 repeated blood tests for drug concentrations over 8 hrs. Frequent blood tests are invasive and require the subject to stay in a medical institution for a long time. In this study, we simulated BET to assess whether these blood tests could be replaced by non-invasive 13C breath tests.

Methods: Randomized 2 experimental zone crossover test. 16 male and 5 female healthy volunteers ingested chocolate coated 13C-enriched sodium acetate (100 mg) as a simulated generic drug, or 13C-enriched sodium acetate alone as a simulated original drug. Breath was sampled at 10, 20, 30, 40, 50, 60, 70, 120, 180, 240, 300 and 360 minutes after the ingestion. This procedure was repeated a week later with the other drug. The CO2 isotope ratio (13CO2/12CO2) of the breath samples were measured using infrared spectrometer and pharmacokinetic parameters (Cmax and AUC) were compared between the drugs.

Results: BET with 13C-breath test provided results equivalent to that with blood test. The intra- and inter-subject variances of the parameters were significantly smaller with the breath test compared to the blood test.

Conclusion: Frequent sampling by non-invasive 13C-breath test provides BET with precision greater than blood test. Application of 13C-breath test to BET reduces the burden of the subjects and is expected to promote development and marketing of generic drugs.
Breath Analysis as a Diagnostic Tool for Pneumonia in Mechanically Ventilated Patients

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Whilst ventilator-associated pneumonia (VAP) in intensive care patients is associated with high mortality and costs, culturing pathogenic organisms from lower respiratory tract samples can take several days to confirm diagnoses. Therefore, a rapid diagnostic test is needed to rule-out VAP in order to reduce the unnecessary use of broad-spectrum antimicrobial agents. Exhaled volatile organic compound (VOC) analysis has the potential to yield such a test.

Breath samples were collected from a total of 96 patients, recruited at five hospitals in Liverpool and Manchester, UK, within 24 hours of suspected VAP. Follow-up samples were collected in patients that remained intubated 48-72 hours after first sample collection. Breath samples, collected on sorbent tubes, were analysed for exhaled VOCs by thermal desorption gas chromatography mass spectrometry (TD-GC-MS). A short-listed target library was created based on previously reported VAP-relevant compounds, comprising 37 compounds.

After screening the dataset against the target library, several compounds were found to correlate strongly, particularly compounds that have been identified as putative markers of inflammation and oxidative stress. 16 individual target compounds were found to be significant predictors of VAP after correction for multiple testing by false discovery rate (p < 0.05). Linear discriminant analysis and logistic regression analysis produced significant models, with internal cross-validation (bootstrapping) suggesting that the models were robust (AUROC 0.72 and 0.79 respectively). Other clinical characteristics were tested, including recruiting centre, and were found not to be significant co-variates. Further work is required in order to identify VOCs that are driving this separation, and to determine their utility as diagnostics markers.
60. Portable Exhaled Breath Condensate (EBC) Metabolomics for Daily Monitoring of Adolescent Asthma

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Background

Portable methods for measurement and quantification of biological analytes have tremendous promise to advance non-invasive personal health monitoring. Exhaled breath metabolomics shares this promise and has been gaining popularity as a non-invasive technique amenable to a vast range of medical uses.

Methods

This work compares breath metabolite abundances in six healthy control subjects and five asthmatic subjects. Exhaled breath condensate (EBC) samples were collected with a novel miniaturized sampler. This device enables breath sample collection in multiple environments, including intensive care units, outpatient clinics, workplaces, and at home. A total of 293 breath samples were collected and analyzed longitudinally, including about 28 samples per subject. EBC was analyzed with liquid chromatography-mass spectrometry (LC-MS) to define specific metabolite differences between subjects. Untargeted and targeted metabolomic analyses were performed simultaneously, but with separate data analysis procedures.

Results

Individual differences among subjects were found longitudinally. When presented by health condition, group differences were enhanced with a clear separation between subjects belonging to either the control or asthmatic group. Unexpectedly, targeted compounds consistently had lower intensities in asthmatics. There is a distinct pattern of a day/night cycle with elevations of peak area values in evening samples. These differences were presented mainly in asthmatic subjects, which can be explained by asthma being a representation of exaggerated amplitudes compared to healthy circadian patterns.

Conclusions

Untargeted and targeted analysis of EBC using this device allows the discovery of novel endogenous metabolic signals in a biological sample and the daily monitoring of selected metabolites related to diseases and medical conditions.
61. Analysis of Volatile Organic Compounds to Predict Hyperbaric Pulmonary Oxygen Toxicity in US Navy Divers

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Background: Hyperbaric oxygen exposure encountered in diving operations utilizing closed circuit oxygen rebreathers is limited by onset of pulmonary oxygen toxicity symptoms, which is a reportable diver mishap. There is significant variance in the presentation of PO2T between individuals, and there is no real-time individualized prediction tool for the onset or resolution of PO2T. A non-invasive biomarker of PO2T would serve to individualize operational decisions for military members working in O2-enriched environments and has the potential to reduce this form of diving mishap. A breath test utilizing exhaled volatile organic compounds (VOCs) might enable the early recognition of PO2T prior to symptom onset. The purpose of this study was to determine a VOC profile predictive of PO2T using human breath samples collected during hyperoxic diving studies at the US Naval Submarine Medical Research Laboratory (NSMRL) and the US Navy Experimental Dive Unit (NEDU).

Methods: Breath samples were collected into 1L ALTEF bags and drawn onto Tenax TA thermal desorption tubes using a MultiRAE pump. The samples were analyzed using gas chromatography-mass spectrometry (GC-MS) coupled to a Markes thermal desorption system. Raw chromatogram files were converted to ELU files using Xcalibur and AMDIS; then SpectConnect was used to find conserved components and biomarkers. The difference of relative abundance for breath and background (breathing circuit) yielded a VOC matrix. This matrix was analyzed to find significant changes between pre- and post-dive. Using SAS software, ROC curves were generated to identify VOCs predictive of PO2T symptoms.

Results: For the NSMRL study, PO2T symptoms (e.g., cough, chest tightness, fatigue) were present during 13 of 168 dives (8%), and GC-MS breath analysis detected 96 VOCs. For the NEDU study, PO2T symptoms were present during 31 of 267 dives (11.6%), and 154 VOCs were detected. Pooling data from both studies, 18 VOCs were found to overlap, of which 7 changed significantly with hyperoxia exposure, and 4 predicted PO2T symptoms with a ROC AUC ranging from 0.63 to 0.75.

Conclusions: This study identified VOCs that predict PO2T symptoms in divers. A second VOC study is ongoing at NEDU to validate and expand upon these findings and create an operational decision making tool for military members working in O2-enriched environments.
Abdominal Center Nephrology; University of Helsinki and Helsinki University Hospital, Helsinki, Finland...123
Adams ER ........................................... 93
Ager C ............................................. 172
Ahmed W .......................... 113, 157, 158
Air Force Research Laboratory; 711th Human Performance Wing/RHXBC, Wright-Patterson AFB, OH, USA... 42, 106
Al-Khalifa Y ................................. 104
Allen L ........................................... 122
Allsworth M ....................................... 91
Altenburg J ...................................... 170
AMP-Lab GmbH, Mendelstr. 11, 48149 Münster, Germany .................... 37
Amsterdam University Medical Centers,
University of Amsterdam - Amsterdam (Netherlands).................. 95, 110, 170
Anatune Ltd, Cambridge, United Kingdom ........................................ 54
Ancon Medical Inc., Bloomington, Minneapolis, Minnesota .......... 120
Ancon Technologies Limited, CIC, University Rd., Canterbury, Kent CT2 7FG, UK; ............................................. 120
Anil Modak ........................................ 92
Avila J ................................................ 32
Avison M ........................................... 149
Azmir J ........................................... 72, 102
Baas P ............................................. 95, 110
Baghdasaryan A ................................ 46
Bähler P ........................................... 46
Bangen LM .......................................... 53
Bannard-Smith J .................................. 177
Barker B M ........................................ 34
Barkowsky G ..................................... 39
Barreiro P ........................................... 63
Bartels J ........................................... 37
Batista GL .......................................... 55
Batty C ................................................ 149
Bauer T ............................................. 139
Bean H D ........................................... 34
Beardsmore C ............................ 61, 145
Beauchamp J .................................... 165, 167
Beccaria M ........................................ 102
Becher G ......................................... 173, 174
BecherConsult GmbH Bernau...173, 174
Beesley K ......................................... 98
Behrendt M ....................................... 128
Bei Sun ........................................... 117
Bekkers M ......................................... 95
Bell KJM ........................................... 54
Bellagambi FG ................................... 52, 83
Berger C ........................................... 169
Bergmann A ...................................... 30
Biagini D ......................................... 52, 83
Bierl. R ............................................. 132
Biomedical Engineering, Basel University, Basel, Switzerland .......... 63
Biomolecular Sciences Research Centre; Sheffield Hallam University, Sheffield, United Kingdom ..................... 122
Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA ........................................... 32
Blakley J ....................................... 87
Blomberg A ....................................... 44
Bobak C ........................................... 102
Boehm C ......................................... 128
Boots A ............................................. 70
Borras E ........................................... 76
Bos LD ............................................ 94, 170
Boullind C ........................................ 98
Bouvy N D ......................................... 36, 112
Breathomix - Reeuwijk (Netherlands). 95, 110
Brenner H ......................................... 79
Brewer P ........................................... 82
Brightling CE .......... 59, 61, 104, 121, 145
Brinkman P ...................................... 170
Brinkman P ...................................... 94, 110
Bristol Urological Institute, Bristol, United Kingdom .................. 149
Brodrick E ........................................ 93
Bromley MJ ....................................... 158
Bröms K .......................................... 58
Brothers MC ............................. 42, 106
Bruderer T ....................................... 46, 67, 144, 169
Bruhn S .......................................... 85
Bryant L ............ 59, 61, 121, 145
Buchanan A ....................... 161, 164
Buszewski B ............ 89, 165, 167
Calcagno M..........................91
Cappellin L..........................144
Cardiovascular Sciences, University of
Leicester..........................145
Carolan V..........................122
Carr L..........................121
Cartwright L..........................91
CAS Key Laboratory of Separation
Sciences for Analytical Chemistry,
Dalian Institute of Chemical Physics,
Chinese Academy of Sciences, Dalian,
People's Republic of China...........117
Center for Comparative Respiratory
Biology and Medicine, University of
California - Davis, Davis CA, USA.....76, 140, 180
Center for Fundamental and Applied
Microbiomics, The Biodesign Institute,
Tempe, AZ..........................34
Centre for Analytical Science,
Department of Chemistry,
Loughborough University,
Loughborough, UK59, 61, 68, 142,
145, 147, 156
Centre of Research in Biosciences,
University of the West of England,
Frenchay Campus, Coldharbour Lane,
BS161QY..........................98
Chair of Aroma and Smell Research,
Department of Chemistry and
Pharmacy; Friedrich-Alexander
Universität Erlangen-Nürnberg,
Erlangen, Germany .............. 165, 167
Chair of Environmental Chemistry and
Bioanalytics, Faculty of Chemistry;
Nicolaus Copernicus University, Torun,
Poland..........................165, 167
CHANTHAPANYA Chanthakhone ......119
Chapovskiy A..........................120
Chaudhuri N..........................113
Cheng A..................................76
Cheng HR..............................27
Clinical and Molecular Metabolism;
University of Helsinki, Helsinki, Finland,
..................................123
Coats T..............................145
Cole L..............................122
College of Life Sciences, Department of
Infection, Immunity and Inflammation,
University of Leicester, University
Road, Leicester LE1 7RH, UK........104
Computer Science Department,
Loughborough University,
Loughborough, UK..................156
Cordell R................59, 61, 104, 121, 145
Covington J................................93, 98
Coyne K..........................109
Cramphin H..........................140
Cristescu SM.........................55
Dagelet JWF..........................95
Dagelet YW..........................94, 110
Dailey LA..........................153
Dalian Institute of Chemical Physics,
Chinese Academy of Sciences, Dalian,
China..........................51, 56, 127
Dallinga J......................70, 115
Dandang Jiang........76, 140, 180
Dark P..........................177
Darnley K..........................156
Dartmouth-Hitchcock Medical Center;
Dartmouth College, Hanover, USA...75
Datta AN..........................110
Davidson CN..........................42, 106
Davis CE..........................76, 140, 180
Davis, MD..........................78
De Jongh F..........................95, 110
de Lacy Costello B........98, 131, 146, 149
De Radigues R......................158
de Vries R.........................95, 110
Deep Breath Initiative AZ, Zug,
Switzerland..........................63
Department of Anaesthesia and Intensive
Care, University Medicine Rostock,
Rostock, Germany..30, 37, 38, 39, 85
Department of Anesthesiology, The First
Affiliated Hospital of Harbin Medical
University, Harbin, China48, 51, 56,
57, 117, 119, 127, 135, 151
Department of Applied Physics and
Electronics, Umeå University, SE-
90187 Umeå, Sweden.................44
Department of Biochemistry and
Molecular Biology, Michigan State
University, East Lansing, MI, 48824 32
Department of Biochemistry and
Molecular Medicine, University of
California Davis, 2700 Stockton Blvd.,
Sacramento, CA 95817..................76
Department of Biochemistry, Institute of
Integrative Biology, University of
Liverpool, Biosciences Building, Crown
Street, Liverpool L69 7ZB, UK.......87

174
Department of Pharmacology, Saitama Medical Center, Kawagoe, Japan .......... 45
Department of Public Health and Caring Sciences, Family Medicine and Preventive Medicine, Uppsala University, Uppsala, Sweden ............ 58
Department of Public Health and Clinical Medicine, Umeå University, SE-90187 Umeå, Sweden ............................. 44
Department of Pulmonology & Thoracic Oncology, Antwerp University Hospital; Belgium........................................ 130
Department of Pulmonology, University Hospital Zurich; Zurich, Switzerland 67
Department of Respiratory Medicine .......................... 94
Department of Respiratory Sciences, University of Leicester, UK ............. 59
Department of Sensory Analytics; Fraunhofer IVV, Freising, Germany .................................................... 165, 167
Departments of Intensive Care Medicine; Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands................................. 94
Dept. of Emergency Medicine and Critical Care, Saitama Medical Center, Kawagoe, Japan ................................ 175, 176
Dept. of Marine and Atmospheric Research, Utrecht University, Utrecht, Netherlands....................................... 55
Dept. of Molecular and Laser Physics, Exhaled Biomarkers group, Radboud University, Nijmegen, Netherlands .......................... 55
Dept. of Pharmacology, Saitama Medical Center, Kawagoe, Japan ............ 175
Dept. of Pulmonology, Semmelweiss University, Butapest, Hungary ....... 175
Desheng Liu ....................................................... 57
Devine T ......................................................... 131
Dhanarisi J ....................................................... 68
Dharmawardana N .............................................. 97
Di Francesco F .............................................. 52, 83
Diagnostiek voor U - Eindhoven (Netherlands) .............................. 110
Dijkers E ....................................................... 95, 110
Discipline of Surgery, College of Medicine and Public Health, Flinders University, Adelaide, South Australia ............ 97
Division Gastroenterology-Hepatology, Department of Internal Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands ........................................ 115
Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Switzerland ..................... 169
Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Switzerland .......................... 46, 67
Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany ............................ 79
Division of Diabetes, Endocrinology and Gastroenterology, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, UK ............................................. 87
Division of Hematology and Oncology, Department of Internal Medicine, 4501 X Street, Suite 3016, Sacramento, CA 95817, USA .................... 76
Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK 87, 113, 134, 142, 147
Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom ........................................ 177
Division of Infectious Diseases, University Children's Hospital Zurich, Switzerland ........................................ 169
Division of Informatics, Imaging and Data Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, UK ............................................. 87
Division of Population Health, Health Services Research & Primary Care, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom ........................................ 177
Division of Preventive Oncology ................................ 79
Division of Proteomics of Stem Cells and Cancer .......................... 79
Division of Respiratory Medicine and Childhood Research Center, University Children’s Hospital Zurich, Switzerland
................................. 46, 67, 144, 169
Division of Rheumatology, Allergy and Clinical Immunology, University of California Davis Health; Sacramento, CA, USA ................. 180
Dongchun Wang.............. 48, 57, 117, 151
Drabinska N...................... 149
Duffy E............................. 129, 154, 155
Durrington HJ..................... 87
Eddleston M...................... 68, 156
Edinburgh Cancer Centre NHS Lothian, Edinburgh, UK ...................... 156
Ehrle S............................. 60
Ekat K.............................. 37
Enyou Li48, 51, 56, 57, 117, 119, 127, 135, 151
ETH Zurich, Department of Chemistry and Applied Bioscience, Zurich, Switzerland
..................................... 46, 67, 144, 169
Eva B.............................. 180
Fabius T............................ 95
Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj Napoca, Romania ...................... 89
Faculty of Science, Technology, Engineering & Mathematics, The Open University, Milton Keynes, United Kingdom ...................... 149
Farzan N.......................... 95, 110
Feasey N........................... 93
Felton T............................ 177
Fischer S......................... 30
Fisher CL.......................... 32
Fitzgerald S....................... 129
Flemish Institute for Technological Research, Unit Health, Industriezone Vlasmee, 2400 Mol, Belgium .... 125, 126
Flynn JL.......................... 72, 102
Focant J-F.......................... 99
Fois M............................. 136
Folkhälsan Institute of Genetics; Folkhälsan Research Center, Helsinki, Finland ...................... 123
Fossil Ion Technology SL, Madrid, Spain ........................................... 63
Fowler SJ81, 87, 113, 134, 142, 157, 158, 177
Francis N.......................... 98
Franciscus Gasthuis en Vlietland-Rotterdam (Netherlands) ...................... 95
Frank M............................ 32, 76
Franzoni........................... 83
Fraunhofer IPMS; Dresden, Germany 139
Fraunhofer ITEM, Bio- and Environmental Analytics, Hannover, Germany ........... 128
Fraunhofer ITEM, Clinical Airway Research, Hannover, Germany ........... 128
Fraunhofer ITEM, Member of the German Center for Lung Research (BREATH), Hannover, Germany ...................... 142
Fraunhofer IZI Leipzig...................... 174
Fraunhofer IZI; Leipzig, Germany ...................... 139
Fraunhofer Project Hub "Microelectronic and Optical Systems for Biomedicine" MEOS, Erfurt, Germany ...................... 53, 139
Free R............................. 59, 104, 145
Frey U.............................. 101
Fukushima K........................ 45
Fuoco R............................. 52, 83
Fusi J............................. 83
G.A.S. Gesellschaft für analytische Sensorsysteme mbH, Dortmund, Germany ...................... 93
Gaillard EA........................ 61, 145
Gandelman O ...................... 91
Gas and Particle Metrology Group; National Physical Laboratory, Teddington, United Kingdom ...................... 82
Gaugg MT.......................... 144
Gawarammana I ...................... 68
Geisel School of Medicine at Dartmouth, Hanover, NH 03755 ........... 75, 102, 108
Genomics and Proteomics Core Facility ...................... 79
Geranios P.......................... 158
German Cancer Consortium (DKTK), Heidelberg, Germany ...................... 79
Ghimenti S.......................... 52, 83
Ghio AJ............................ 153
Ghorbani R ....................... 44
Gierschner P ....................... 38
Goodacre R........................ 87, 158, 177
Goossens R ....................... 125
Gorbunov B ....................... 120
Gould O........................... 98
Graduate School of Life and Health Sciences, Chubu University, Aichi, Japan ...................... 150
Graf A.............................. 139
Institute of Medical Microbiology, Virology and Hygiene, Rostock University Medical Center, Schillingallee 70, 18057 Rostock, Germany........................39
Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany........................................30, 38
Institute of Sport Science, University of Rostock, Ulmenstrasse 69, 18057 Rostock, Germany..........................85
Interdisciplinary Centre for Modern Technologies, Nicolaus Copernicus University, Torun, Poland...................165
IONICON Analytik, Innsbruck, Austria........................40
ISX and interscience, Breda, The Netherlands.......................99
Jaeschke C.................................................................159
Jak PMC........................................................................95
Janson C ........................................................................58
Janssen H ........................................................................125
Janssens E .........................................................................130
Jessa Hospital, Pediatrics, Stadsomvaart, 3500 Hasselt, Belgium ...125
Jessa Hospital, Pediatrics, Stadsomvaart, 3500 Hasselt ..............126
Jinghui Shi ......................................................................57
Jinno N .............................................................................133, 150
JLM Innovation GmbH, Tuebingen, Germany........................159
Joint Medical Center Arabkir, Division of Pulmonology, Yerevan, Armenia ....46
Jones AD ........................................................................32
Jonkers D ........................................................................27, 115
Joyce A .............................................................................68
Junghans T ........................................................................174
Kamp G .............................................................................37
Kang L .................................................................................75, 109
Kasbohm E ........................................................................38
Kenyon NJ .........................................................................140, 180
Kepler E H .........................................................................34
Kester S ..............................................................................95
Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, People’s Republic of China........................151
Khan MS ..........................................................................102, 109
Kikuste I .............................................................................159
Kinderartsen Huis 5, Pediatric practice, Stadsomvaart, 3500 Hasselt, Belgium ........................................126
Klemenz A-C .................................................................37, 39
Klinikum Bayreuth, Bayreuth ....................................174
Koenemann M ...............................................................40
Köhler H ...........................................................................30, 38
Kohler M .............................................................................67
Kondo T .............................................................................150
Koppen G ..........................................................................125, 126
Kox M ...............................................................................112
Kriekemeyer B .....................................................................39
Krilaviciute A .......................................................................79
Kuhlmeier D ......................................................................53, 139
Küntzel A .............................................................................38
Lab. Experimental Medicine & Pediatrics, Antwerp University ................130
Laboratory for Environmental and Life Sciences, University of Nova Gorica, Nova Gorica, Slovenia ...............177
Laboratory for Environmental and Life Sciences, University of Nova Gorica, Vipavska 13, SI-5000 Nova Gorica, Slovenia ................................................87, 134, 142
Laboratory of Pediatrics, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands ..................70
Lammers A ..........................................................................94
Lamote K ..........................................................................130
Lane TW.............................................................................32
Langford VS .......................................................................54
Lehmann U .........................................................................41
Lehto M ............................................................................123
Lei Guo ...48, 51, 56, 57, 119, 127, 135
Lei Hua ..............................................................................51, 56
Leicester NIHR Biomedical Research Centre (Respiratory theme), Glenfield Hospital, Groby Road, Leicester LE3 9QP, UK ................................................104
Leijte GP ..........................................................................112
Leja M .................................................................................159
Leonard C ..........................................................................113
Liebscher V ........................................................................30, 38
Ligor T ..............................................................................89, 165, 167
Lin Cui ...............................................................................57, 119, 135
Lin PL ...............................................................................72, 102
Lindekens J .........................................................................126
Linlin Ji .............................................................................135
Lisspers K ..........................................................................58
Little LD .............................................................................122
Liverpool School of Tropical Medicine, Liverpool, UK .................................................................93
Lomonaco T .......................................................................52, 83
Loudon A .............................................................................87
Louis R ........................................ 99
Lubbers T .................................. 36, 112
Lucas A .................................... 110
Lyu Yang .................................. 135
Macia, M .................................. 63
Madden MC ................................ 153
Maguire P ................................ 137
Maidstone R ................................. 87
Maitland-van der Zee AH ............ 170
Maitland-Van Der Zee AH .... 94, 95, 110
Majoer CJ .................................. 170
Mallinson A ................................ 38
Manek A ................................... 165, 167
Manchester Academic Health Science Centre ..................... 81
Manchester Academic Health Science Centre and NIHR Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, UK .................................. 87, 134
Manchester Academic Health Science Centre, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom ...... 177
Manchester University Hospitals NHS Foundation Trust, Wythenshawe, UK .................................. 142, 157, 158
Manek A ................................... 68
Mansouri TS ................................ 137
Markes International, Llantrisant, UK .................................. 161, 162, 163, 164
Martin H .................................. 162, 163
Martin JA .................................. 42, 106
Materic D .................................. 55
Mathematical Sciences Department; Loughborough University, Loughborough, UK .................................. 156
Matsueda H ................................ 175
Matysik FM ................................ 132
Mayhew CA ................................ 91
McCortney MM .............................. 140
McGregor L ................................ 161, 164
McLaren D B ................................. 156
McNally T .................................. 61
Mead H ..................................... 34
Mechanical and Aerospace Engineering, University of California - Davis, Davis CA, USA .............................. 140, 180
Median Kliniken Heiligendamm, Germany .............................. 173
Medical Center Den Bosch Oost – Den Bosch (Netherlands) ......................... 95
Medical Research Triangle Park, NC, 27709, USA .............................. 66
Medisch Spectrum Twente - Enschede (Netherlands) ......................... 95, 110
Mellors TR ................................ 72, 102
Member of the German Center for Lung Research (BREATHE), Hannover, Germany .............................. 128
Meng Li .................................... 48, 57, 119
Meoli SD .................................. 42, 106
Metsäälä M ................................ 123
Meyer J ....................................... 37
Micc S ....................................... 46, 169
MICROSENS SA, Lausanne, Switzerland .................................. 41
Miekisch W ............................. 26, 30, 37, 38, 39, 85
Milanowski M .............................. 89
Miles L ..................................... 162, 163
Mitrovics J ......................... 159
Mizarakoff B ................................ 60
Moeller A .................................. 169
Möller A .................................. 46, 67, 144
Mondeiro F ................................ 89
Monks PS ................................. 61, 121, 145
Moreno S .................................. 82
Morrin A .................................. 74, 129, 154, 155
Muala A .................................. 44
Mueller D .................................. 120
Mueller T .................................. 79
Mujagic Z .................................. 27
Müller S .................................. 67, 169
Nagahama Institute of Bio-Science and Technology, Shiga, Japan .............................. 150
Nagamine K ................................ 141
School of Informatics and Sciences .............................. 141
Nailon W H .............................. 156
National Center of Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27709, USA .............................. 66
National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27709, USA .............................. 66
National Institute of Health Research (NIHR) Leicester Biomedical Research Centre (BRC), UK .................................. 145
National Jewish Health, Denver ............ 108
Neerincx AH ........................................... 94, 170
Netherlands Cancer Institute - Amsterdam (Netherlands) ........... 110
Ng L .................................................. 145
Nguyen AP ........................................... 180
Ngyugen AP ........................................... 140
NIBEC - Nanotechnology and Integrated Bio-Engineering Centre University of Ulster, Newtownabbey, Co Antrim, Northern Ireland .................. 137
NIHR Biomedical Research Centre (Respiratory theme) and Department of Respiratory Science, University of Leicester, Leicester, UK .......... 121
NIHR Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, Manchester, UK .. 81
NIHR Oxford Biomedical Research Centre, John Radcliffe Hospital, Oxford, UK and Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK ...... 87
North West Lung Centre, Manchester University NHS Foundation Trust, Manchester, UK .................................. 113
Norwich Medical School, University of East Anglia, Norwich, UK ........ 113
Nutricia Research, Utrecht, The Netherlands ........................................... 115
Nuyens C .............................................. 126
of Biology, Medicine and Health, University of Manchester ........... 81
Okumura N ............................................ 133, 150
Onor M .................................................. 52
Orf I ...................................................... 91
Organic and Biological Analytical Chemistry Group, MolSys, University of Liège, Belgium ........................................... 99
Owlstone Medical Ltd., Cambridge, UK .............................. 91
Padayachee D ........................................... 54
Padilla M .................................................. 159
Pako J .................................................... 175
Palangasinghe C ....................................... 68
Parker A .................................................... 161, 164
Parmar A .................................................... 145
PAS Technology Deutschland GmbH, Richard-Wagner-Str. 10, 99441 Magdala, Germany ........................................... 60
Patenge N .................................................... 39
Pearson M .................................................... 68
Peppermint Consortium ........................................... 142
Perkins N .................................................... 46, 67, 169
Persad R .................................................... 149
Peters K ..................................................... 37
Pham Y ..................................................... 165, 167
Pharmaceutical Analysis, KU Leuven, Oude Markt 13, 3000 Leuven, Belgium ........................................... 102
Pharmacology, Toxicology & Therapeutics Unit, University of Edinburgh, Edinburgh, UK .... 68, 156
Pitsch RL ............................................... 42, 106
Pleij JD ...................................................... 66, 153
Polaka I ...................................................... 159
Pugliese G .................................................... 85
Pulse Health LLC, Portland OR, USA ........................................... 140
Purkhart R ................................................... 174
Pussinen P ................................................... 123
Qi Li ......................................................... 135
Radboud University Medical Center - Nijmegen (Netherlands) .................. 95
Radermacher P ............................................. 60
Radic M ...................................................... 93
Raes M ...................................................... 125, 126
Rasley A .................................................... 32
Ratcliffe N .................................................... 98, 131, 146, 149
Ratiu IA ..................................................... 89
Ray DW ....................................................... 87
Read ND .................................................... 158
Redknight Consultancy Ltd, Abercynon, UK ........................................... 93
Redlberger S .................................................. 30
Rees CA ..................................................... 75
Reese KL .................................................... 32, 76
Reinhold P .................................................... 30, 38
Repp S ......................................................... 60
Respiratory Medicine, GIGA I3, CHU Sart-Tilman, University of Liège, Belgium 99
Richards LB .................................................. 94
Richardson M .............................................. 104, 121
Riga State Gymnasium No.3, Riga, Latvia ........................................... 159
Risby TH .................................................... 140
Roberts SA .................................................... 177
ROMBAT, Department of Anesthesiology and Intensive Care Medicine, University Medical Centre Rostock, Germany ........................................... 26
Roslund K .................................................... 123
Ruszkiewicz D 59, 68, 104, 142, 145, 147
Rutter A .................................................... 120
Yan Wang........................................51
Yang Li ......................................51, 127
Yang Lv ......................................48, 127
Yang Lyu ......................................57
Yazbeck R .....................................97
Yeap D.........................................180
Yeovil NHS Trust, Higher Kingston, Yeovil, BA21 4AT.................................98
Yinghua Cui..................................57
Yiping Liu ..................48, 56, 117, 119, 151
Yousuf A......................................121
Yuanyuan Xie.................................56
Zanella D .....................................99
Zenobi R .................................46, 144, 169
Zhao B .................................59, 104, 121, 145
Zhongzhi Qiu .................................48, 57
Ziesenitz V ..................................101