# International Conference on Oral Malodour 2 (ICOM2)

# Bristol, UK

# Conference Programme

# 02-04 July 2023

# Welcome from the Hosting Institution Organisers

Dear Delegates,

We are delighted to welcome you to the 2nd International Conference on Oral Malodour (ICOM2). If you came to our first conference in 2019 you remember that we planned to meet annually/biannually. Then of course COVID-19 came, which put a stop to any oral malodour research for the past few years. It is fantastic to see that our research, despite the huge impact of the pandemic, has now bounced back.

We are very grateful to you for coming, some of you from far away, and attending this very important event. I would also like to thank you for supporting oral malodour research and for presenting your work. I hope that this meeting will be very successful and drive us to continue our search for new ideas, approaches and improvement of our current methods used for oral malodour diagnosis and treatment. This will be another excellent opportunity to engage with a range of scientists and researchers, some very experienced in the field and others new to the subject area of oral malodour. I hope that this event will encourage each of us to get together more often to keep this area of research very active and sustainable for many years to come.

Finally, I would like to thank our sponsors for their generosity in supporting this conference. I also address my sincere appreciation to the University of the West of England for their support in the realisation of this event.

Saliha Saad - Principal Organiser

**Local Organising Personnel (Bristol)**

Debbie Lewis

Eleri Heathcote

# Sponsors








The ICOM2 2023 Conference wishes to give special recognition to the sponsors who have made this event possible.

We also thank the **Centre of Research in Biosciences at UWE Bristol** for financial support, time and effort in coordinating this event.

# Conference Objectives

This conference will give updates on the latest developments in oral malodour research. It will be an excellent opportunity to discuss with oral malodour experts their invaluable experience in the field and their vision for the future of oral malodour research. This conference will be an ideal opportunity to share knowledge, make new connections with, research scientists, industry partners, engineers, and clinicians. The influence that Covid-19 epidemic had on conducting oral malodour research will be debated, and the way forward to arm us in the event of another epidemic discussed. Finally, this conference intends to publish the best papers through special issue by the Institute of Physics Publishing and the Journal of Breath Research.

# Key Themes

1. The sense of smell, its physiology and physiopathology
2. Oral malodour, oral diseases diagnostic and control
3. The oral microbiome and its link to systemic diseases
4. Metagenomics and oral diseases
5. Microbial physiology
6. Analytical tools for non-invasive diagnosis of disease
7. Development of point-of-care instruments for early diagnosis of disease
8. An open discussion on the safety of the organoleptic assessment following Covid-19 pandemic and a consensus on the safest method to use

# Key Event Information

**Dates**
The conference begins at 16:00 on Sunday the 02 July and closes at 14:00 on Tuesday the 04 July.

**Venue**
De Vere Tortworth Court
Tortworth
Wotton Under Edge
GL12 8HH
Tel. no. +44 (0) 1454 263000

**Registration Information**
Name badges and final programs will be distributed at the Registrations Desk located in the Westminster Lobby (located at front of hotel).

Registration Desk Hours:

| | |
|---|---|
| Sunday 2nd July | 14:00-18:00 |
| Monday 3rd July | 08:00-09:00 |

**Refreshments**

- Tea, Coffee and biscuits will be provided every morning and afternoon break in the Orangery.
- A BBQ will be provided in the Orangery from 19:00, 02 July 2023. Other drinks can be paid for at the bar.
- Lunch will be served daily in the restaurant.
- An evening meal is provided at 20:00 on the evening of the 03 July 2023 in the Orangery.

**Information for Presentations**

- Please provide a copy of your presentation at registration by USB pen drive (or before conference begins to Debbie.lewis@uwe.ac.uk).
- Please name your file using the following format: Day of presentation-last name.ppt e.g. Tuesday-Lewis.ppt
- Only Powerpoint or PDF presentations will be accepted.
- All presentations will be given using the computers provided by the venue.

# Conference Programme

| **02 July** | | |
|---|---|---|
| | **Room** | **Activity** |
| 16:00 -16:10 | Brunel Room | Welcome from Host Organiser – Ass. Prof. Saliha Saad |
| 16:10- 16:20 | | Welcome from Pro-Vice Chancellor and Head of College of Health, Science and Society, UWE Bristol – Prof. Marc Griffiths |
| 16:20-16:35 | | Welcome from the Director of the Centre for Research in Biosciences, UWE Bristol – Prof. Aniko Varadi |
| 16:40-17:10 | | Oral malodour research will never die – Prof. Mel Rosenberg |
| 17:15-17:45 | | Why is smell important? How does it work? What happens when we lose our sense of smell? How can it be restored? Prof. Thomas Hummel |
| | | |
| 18:00-19:00 | | Pre-event Drinks |
| 19:00 onwards | | BBQ |
| | | |
| | | |
| **03 July** | | |
| | | **Chair:- Prof. Aniko Varadi** |
| | | **Session Theme: Diagnosis and Treatment** |
| 08:30-09:00 | Brunel Room | Halitosis diagnosis: a questionnaire study among specialists – Dr Isabelle Laleman |
| 09:05-09:35 | | Treatment of halitosis, a difficult escape - Dr Yves Reingewirtz and Dr Ariela Carno |
| 09:40-10:00 | | Oil Pulling Mouthwash as a Natural Alternative to Chemical Mouthwashes: An exploratory study – Dr Sushma Nachnani |
| 10:05-10:35 | | Nanotechnology and the Control of Oral Disease – Prof. Rob Allaker |
| | | |
| **10:45-11:10** | Restaurant | **BREAK** |
| | | |
| | | **Chair:- Prof. Tony Killard** |
| | | **Session Theme: Oral Microbiome and Systemic Conditions** |

| | | |
|---|---|---|
| 11:15-11-45 | Brunel Room | Oral dysbiosis level in the elderly: clinical and laboratory evaluation of subjects living in the industrial and "blue zone" areas on Sardinia Island – Dr Germano Orru |
| 11:50-12:20 | | The interplay between periodontitis and chronic kidney disease – Prof. Mike Curtis |
| 12:25-12:55 | | Are bacteria good for your brain? Linking microbes, their metabolites, and the brain's defences – Prof. Simon McArthur |
| | | |
| **13:00-13:55** | Brunel Room | **LUNCH** |
| | | |
| | | **Chair:- Dr Isabelle Laleman** |
| | | **Session Theme: Metagenomics** |
| 14:00:14:30 | Brunel Room | What insight can metagenomics offer regarding the development and progression of oral diseases? – Prof. David Spratt |
| 14:35-15:05 | | The Oral Microbiome Unmasked: Microbiome Sequencing as a Tool for Understanding Oral Health Oral Health _ Jegor Oleinikov |
| 15:10-15:40 | | Functional potential of different oral niches to contribute to oral malodour – Dr Abish Stephen |
| | | |
| **15:45-16:10** | Restaurant | **BREAK** |
| | | |
| | | **Chair:- Romain Dubreuil** |
| | | **Session Theme: Microbial Physiology** |
| 16:15-16:45 | Brunel Room | The stability of the tongue biofilm parameters over a year period – Prof. John Greenman and Ass. Prof. Saliha Saad |
| 16:50-17:20 | | Nitrate-enrichment reduces volatile sulfur compounds and activates the nitrate-nitric oxide pathway in salivary and anaerobic bacteria associated with human halitosis - Dr M. Tian, Christine Da-Ruh Wu |
| 17:25 – 18:30 | | "To sniff or not to sniff, that is the question" An open discussion, round table on the effect of Covid-19 on practicing the organoleptic assessment by odour judges – Ms. Pat Lenton and Ass. Prof. Salih Saad |
| | | |
| 20:00 - late | Orangery | **CONFERENCE DINNER** |

| **04 July** | | |
|---|---|---|
| | Brunel Room | **Chair:- Dr Sushma Nachnani** |
| | | **Session Theme: Analytical approaches** |
| 09:00-09:30 | | Validation of ammonia breath sensors using SIFT-MS – Dr Tony Killard |
| 09:35- 10:35 | | Considerations on confounding factors in sampling breath volatiles: uptake and emissions by sampling materials – Dr Jonathan Beauchamp |
| 10:10-10:40 | | Exhaled breath real-time analysis for halitosis research – Dr Phillipe A Guy |
| | | |
| **10:45-11:10** | Restaurant | **BREAK** |
| | | |
| | Brunel Room | **Chair:- Dr Abish Stephen**<br>**Session Theme: Volatolomics** |
| | | |
| 11:15- 11:35 | | The effect of viral filters on the volatile profile produced by bacterial biofilms<br>– Dr Keith Hewett |
| 11:40- 12:20 | | Ammonia as a biomarker for health application. Early investigation using a Mach-Zehnder Interferometer optical nose – Dr Theirry Livache |
| 12:15 -12:45 | | Investigating the Headspace of Biofilms with an Optical Nose to Pave the Way for Point-of-Care Diagnostic – Dr Romain Dubreuil |
| 12:50-13:00 | | Closing Remarks – Ass. Prof Saliha Saad |
| | | |
| **13:00-14:00** | Restaurant | **LUNCH** |
| | | |
| | | **CONFERENCE END** |
| | | |

**The poster exhibition will be available to view throughout the conference in the Orangery.**

Many thanks for your participation and attendance.

# Oral Abstracts

## Oral dysbiosis level in the elderly: clinical and laboratory evaluation of subjects living in the industrial and "blue zone" areas on Sardinia Island

**Germano Orrù[1], Cristina Demontis[1], Flavio Cabitza[2], Sara Fais[1], Cinzia Casu[1] and Alessandra Scano[1]**

[1]Department of Surgical Sciences -Oral Biotechnology Laboratory (OBL) -University of Cagliari, Cagliari Italy [2]Fondazione della tutela dell'identità Ogliastrina.

### Introduction

A "blue zone" is a region where people tend to live noticeably longer and are healthier. One of the five Blue Zones identified by scientists throughout the world is located inside Sardinia Island, which has a high percentage of men and women who live to be over 100 years old. At the same time, following recent studies, the host's state of health and the makeup of the oral microbiome are strictly interconnected. In fact, research in molecular microbiology has lately concentrated on a few potentially pathogenic oral anaerobic bacterial species, known as periodontal bacteria or oral pathobionts (PB). The PBs are the cause of severe oral diseases, but they may also contribute to the development of a number of chronic systemic illnesses, including intestinal cancer, rheumatoid arthritis, neurological disorders, and others. For this reason, inside the oral microbiota, the PBs must be represented with a low titer and/or with no significant pathogenic genotypes. In this work, we have compared the oral biosis status of elderly subjects living in two different Sardinian regions: (i) Ogliastra, in the Blue Zone area, and (ii) Cagliari City, characterised by a highly industrialised region already 100 years ago. The hypothesis of this study is that the oral microbiota composition between these two different courts could be different, with a major dysbiotic status in the subjects living in the industrial area compared to the blue zone elderlies, and that this different microbial profile could be associated with life length and health conditions.

## Methods

Microbes
Representative oral pathogens already described by SS Sonkrasky in the red and orange groups 1, were detected and quantified by a real-time PCR procedure using molecular beacon probes. The percentage value, of the total bacteria, in the genomes of Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium nucleatum, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans, was evaluated in the dental biofilm of elderlies recruited in BZ and in the industrial area.

## Samples

Dental biofilms from BZ and industrial areas were recruited from two main groups: (a) contemporary living individuals (modern group) and (b) historical individuals composed of dental calculi buried in an ossuary in the early 1900s. A total of 50 samples from 85-105 years hold subjects.
Sampling procedure, DNA extraction method and molecular analysis procedure were already described in our previous work2.
Results
We contrasted the oral bacterial profiles of BZ-belonging subjects and industrial area living persons, and we found a significant $\simeq$ 100-fold increase in the second zone for microbes belonging to the Red complex bacteria, which are frequently linked to systemic and oral chronic diseases. On the other hand, this difference is significantly reduced as we compare the samples of historical subjects recruited in both living areas.

## Conclusions

These preliminary findings point to a rise in oral PB pathogen markers as a result of the difference in lifestyle between these two housing areas, especially in the alimentary patterns, i.e., the prevailing use of functional antioxidant-rich food in the Sardinia BZ.

**References**

1. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998; 25:134-44.
2. Casula E, Contu MP, Demontis C, Coghe F, Steri GC, Scano A, Ferrando ML, Orrù G. Changes in the oral status and periodontal pathogens in a Sardinian rural community from pre-industrial to modern time. Sci Rep. 2022; 12:15895.

## Nanotechnology and the control of Oral Diseases

**Professor Rob Allaker, BSc (Hons), PhD, FHEA, FISAC**

Queen Mary University of London

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometre scale. The potential of nanoparticles to control the formation of oral biofilms, as a function of their biocidal, anti-adhesive, and delivery capabilities, is under investigation. Nanoparticles as constituents of prosthetic device coatings, topically applied agents, and within dental materials are being explored. The antimicrobial activities of metal / metal oxide nanoparticles and their composites against oral pathogens associated with peri-implantitis, periodontitis and malodour were examined under anaerobic conditions. The activity of the nanoparticles evaluated in descending order was Ag > Ag+CuO > $Cu_2O$ > CuO > Ag+ZnO > ZnO > $TiO_2$ > $WO_3$. Time–kill assays with ZnO demonstrated a significant decrease in growth of all species tested within 4h, reaching 100% within 2h for *Porphyromonas gingivalis*. Antibiofilm activity of nanoparticulate coated titanium discs were investigated using a constant depth film fermenter (CDFF)-based polymicrobial biofilm system. Nanoparticles of zinc oxide (nZnO), hydroxyapatite (nHA) and a combination (nZnO+nHA) were coated using electrohydrodynamic deposition onto Ti discs.

Using human saliva as an inoculum, biofilms were grown on coated discs for 96h under aerobic conditions with artificial saliva and peri-implant sulcular fluid. The proportion of non-viable microorganisms was shown to be higher on nZnO and composite coated surfaces at 96h compared with nHA coated and uncoated titanium. Biofilm thickness comparison also demonstrated that nZnO and composite coatings to be the most effective. Related osteoblast proliferation and differentiation studies revealed that substrates coated with nZnO alone were more efficient than composite surface coatings. Novel copper-bearing titanium alloys (Ti-Cu) have also been examined as regards Cu ion release and antibiofilm activity. Results from gene expression studies, biofilm growth observation, bacterial viability measurements and morphological examination of bacteria, revealed antimicrobial/antibiofilm activities of Ti-Cu alloys against *P. gingivalis* and *Streptococcus mutans*. Increased transcription of pro-osteogenic and Cu transporting genes were also observed in osteoblasts exposed to Ti-Cu.

In conclusion, although metals are known to suppress the growth of VSC-producing and other oral bacteria, and to inhibit malodour through direct binding to hydrogen sulphide, translational challenges remain as regards the *in vivo* use of metallic nanoparticles.

## Treating halitosis: A difficult escape

**Dr Yves Reingewirtz and Dr Ariela Carno**

CHU de Strasbourg, Strasbourg, France

Patients with halitosis of oral origin primarily present features which can be divided into similar clinical parameters and a common medical pathway. From those observations one can develop a strategy based on each of those two categories.

The current clinical treatment is appropriate for a health periodontium, corresponding to a Stade I or II Grade A or B. In this condition, treatment will consist of the reduction of the bacterial load by local debridement. It will be completed by the debridement of the dorsum of the tongue which will include mechanical rearrangement, chemical disinfection with a deodorizing solution and nutritional prescription. The choice and dosage of those products is of importance as they are necessary to maintain an appropriate homeostatic balance between the periodontal and lingual microbiota.

The common medical presentation of these patients also explains the numerous emotional disorders which can be observed concomitantly: Stress, depression, anxiety and low self-esteem. Hence, a rigorous but empathic framework has been proposed to allow for the re-establishment of the self confidence that would have been damaged due to the social and emotional consequences of halitosis.

This therapeutic approach will have a set time at each consultation, and the approach will promote a therapeutic environment and safe space for the patient to share information. Since COVID and lockdown, VSCs evaluation has been performed remotely with a well-adapted communication device. Finally, treatment recommendations will be promoted by providing each patient with a structured daily schedule in order to create a secure treatment time frame.

## Validation of Ammonia Breath Sensors using SIFT-MS

**Michal Wagner[1], Saliha Saad[2] and Anthony J. Killard[1,2]**

[1]BreathDX Ltd., Bristol, United Kingdom.
[2]Centre for Research in Biosciences (CRIB), School of Applied Sciences, University of the West of England, Bristol BS16 1QY, United Kingdom.

Ammonia in breath is associated with several potential conditions such as liver and kidney dysfunction, urea cycle disorders and organic acidaemias. The measurement or monitoring of breath ammonia may have potential in the diagnosis and management of such conditions [1-3].

Breath ammonia is challenging to measure with simple instrumentation, while achieving the sensitivity required in breath (≤50 ppb) and selectivity from interferents, particularly humidity. A sensor technology based on polyaniline nanoparticles has been developed which has this potential [4,5]. Low-cost sensors can be mass produced while achieving the sensitivity and reproducibility required. However, validating the performance of such sensors is, itself challenging due to limitations in the availability of suitable instrumental techniques. SIFT-MS is an effective technique for the measurement of trace concentrations of volatiles in gases, including breath, and has ben designed for the sampling of breath for this purpose.

Sensors were developed and validated using SIFT-MS in combination with an artificial breath system [6]. SIFT-MS was used to validate the artificial breath system with respect to first principles. Following validation in artificial breath, sensors were compared with responses from SIFT-MS in a cohort of healthy human volunteers (n=14). The sensors (n=2) showed excellent correlation with SIFT-MS (n=3). Breath measurements were found to be in the range of 100-700 ppb, consistent with previous spectroscopic studies [7] with correlation coefficients of 0.782 (Pearson) and 0.873 (Spearman). Additional measurements of both mouth-sampled air versus exhaled air using SIFT-MS showed no significant differences, suggesting that exogenous bacterial oral ammonia was not a significant contributor to exhaled breath ammonia concentrations.

These sensors and system have the potential to be deployed in point of care applications for breath ammonia measurement in several diagnostic screening and monitoring applications.

**References**

1. Hibbard, T., and Killard, A.J. (2011). Breath ammonia analysis: Clinical application and measurement. Crit. Rev. Anal. Chem. 41: 21-35.
2. Brannelly, N.T., Hamilton-Shield, J.P., Killard, A.J. (2016). The measurement of ammonia in human breath and is potential in clinical diagnostics. Crit. Rev. Anal. Chem. 46(6):490-501.
3. Hibbard, T., Crowley, K., Kelly, F. Ward, F., Holian, J., Watson, A., Killard, A.J. (2013). Monitoring haemodialysis patients at the point of care with a breath ammonia measurement device based on printed polyaniline nanoparticle ammonia sensors. Anal. Chem., 85: 12158-12165.
4. Ngamna, O, Morrin, A., Killard, A.J., Smyth, M.R., Wallace, G.G. (2007). Inkjet printable polyaniline nanoformulations. Langmuir. 23(16):8569-8574.
5. Hibbard, T., Crowley, K., Killard, A.J. (2013). Direct measurement of ammonia in simulated human breath using an inkjet-printed polyaniline nanoparticle sensor. Anal. Chim. Acta, 779: 56-63.
6. Hibbard, T., Crowley, K., Shahbazian, Z., and Killard A. J. (2012). A system for the continuous generation of simulated human breath containing trace gases. Anal. Meth. 4: 2172 - 2176.
7. Hibbard, T., and Killard, A.J. (2011). Breath ammonia levels in a normal human population study as determined by photoacoustic laser spectroscopy. J. Breath Res. 5: 037101.

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## Considerations on confounding factors in sampling breath volatiles: uptake and emissions by sampling materials

**Y Lan Pham[1,2], Wojciech Wojnowski[3,4], Olaf Holz[5,6] and Jonathan Beauchamp[1]**

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[2] Chair of Aroma and Smell Research, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[3] Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland
[4] Department of Chemistry, University of Oslo, Oslo, Norway
[5] Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, 30625 Hannover, Germany.
[6] Member of the German Centre of Lung Research DZL (BREATH), Hannover, Germany.

### Abstract

Sampling approaches for breath (odour) analysis require the use of suitable components for interfacing the patient with the breath collection system. Most importantly in relation to patient safety, the parts should provide a sterile barrier that reduces the risk of transmission of pathogens between patients. Additionally, the sampling interface materials must exhibit low emissions of volatiles to reduce health risks to patients through inhalation exposure, as well as limit the extent of confounding factors in oral malodour datasets by ensuring sample authenticity.

A novel approach to analyse dynamic emissions from interface materials in real-time was developed by coupling a microchamber/thermal extractor (µ-CTE) to a proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOFMS). This configuration allowed the volatile emissions from model 3D-printed samples using different polymer resin materials – subsequently used to print mouthpiece adapters – to be analysed in order to assess their biocompatibility. Further, the uptake from adsorption onto the material surfaces of a variety of representative compounds commonly present in the human breathome was investigated to ascertain potential losses in sample composition.

This talk will report on a novel approach to analyse volatile emissions and uptake of breath volatiles in relation to different breath sampling interfaces and will highlight the influence of different material treatment procedures on the extent of how such emissions/uptake affect breath volatile datasets. Overall, the choice and treatment of sampling materials represents an important consideration in quality assurance in breath (odour) analysis.

**What insight can metagenomics offer regarding the development and progression of oral diseases?**

**Prof David Spratt,**

UCL Eastman Dental Institute

The Human microbiota is complex and plays critical roles in human physiological and psychological processes. The oral microbiota plays a role in numerous oral diseases but characterising it has proved challenging. Traditionally, we have only been able to grow 50% of the community. The advent of molecular biology in the mid 90s meant that culture independent techniques could be used to characterise the whole community. This was, however, slow and expensive. The paradigm shift has been the advent of high throughput "next generation" sequencing and has allowed metagenomic studies to be cost effective as it allowed faster turnaround, bigger cohorts, views into the tail and indeed information on biochemistry and metabolic processes. Both amplicon and shotgun sequencing have made inroads into defining the community more fully. They have identified a huge range of new taxa that comprise the oral community but have largely re-affirmed our view of the community. The presentation will explore whether these massive technological leaps have provided useful insight into oral health and disease and perhaps importantly shed light on new treatment strategies.

## Halitosis: the diagnostic process in specialized halitosis clinics: a questionnaire study

**I. Laleman**

Ku Leuven, Belgium

**Aim:** Gather practice-based information concerning the diagnosis of halitosis in specialized halitosis clinics and the impact of COVID-19 on this.

**Methods:**

This study was based on an online questionnaire consisting of 20 questions about the diagnosis of halitosis. The questionnaire was dispersed to personal contacts of the author, authors of publications on halitosis and the members of the *Associação Brasileira de Halitose.*

**Results:**

80 halitosis specialists answered this questionnaire between May and October 2022. Most respondents were dentist/dental specialists (88%), furthermore there were ENT-doctors, a gastroenterologist, and a clinical psychologist among the respondents. More than one third of the respondents did carry out a special halitosis consultation and is active in scientific research in this field (29/80), 33 are solely active in a halitosis consultation and 18 solely in halitosis research.

When describing the diagnostic process more than 85% of the specialists answered that medical, dental and halitosis history should be a part of this, in addition to an exam of the teeth, gums and the tongue. For 84% of the respondents an organoleptic exam is indispensable and for 69% it is necessary to do an instrumental exam. The ENT exam was answered as the least necessary in this questionnaire with one third of the respondents who considered it necessary.

**Conclusion:**

The diagnostic process of halitosis is a multi-step procedure consisting of a good medical/dental anamnesis and an evaluation of the breath odor.

## Functional potential of different oral niches to contribute to oral malodour

**Dr. Abish Stephen, BSc, MRes, PhD**

Institute Of Dentistry, Queen Mary University of London

Oral malodour is associated with periodontal disease, poor oral hygiene, tongue debris, deep caries lesions, defective restorations, endodontic lesions, and low salivary flow. The tongue is thought to be the main site involved in oral malodour by contributing to the production of malodorous volatile sulfur compounds (VSCs). The tongue has a significantly different microbiome than dental plaque, and we have shown that the tongue microbiome shares more microbial species with dental plaque in gingivitis and periodontitis than health.

These ecological shifts in disease can contribute to malodour. Samples of saliva, tongue biofilm, supragingival, subgingival and interdental plaque from individuals who had healthy oral cavities, gingivitis or periodontitis were incubated with cysteine or methionine substrate to detect the potential of the different niches to produce VSCs in the context of health and disease, along with profiling the microbiome in these niches via 16S rDNA amplicon sequencing. Further, subgingival VSCs were also detected via headspace analysis of samples to ascertain the VSCs being produced in these sites. Almost all samples could produce hydrogen sulfide from cysteine across all niches, compared to methanethiol from methionine which was detected more frequently in the periodontal niches across health and disease than tongue, with saliva incubation also showing presence of methanethiol. As a function of total bacterial load quantified in the different niches, supragingival plaque from the facial and interdental surfaces showed clearly elevated $H_2S$ production in gingivitis and periodontitis than health, with the subgingival plaque showing the highest $H_2S$ production across all niches indicating that greater proportion of bacteria in these niches can metabolise cysteine and methionine than other niches.

The $H_2S$ produced in all niches were correlated with amounts of protein as detected in the samples, with the dental plaque $H_2S$ more strongly associated with amount of protein present in the samples and bacterial species alpha diversity in gingivitis and periodontitis than health. The study highlighted the potential role of periodontal niches in contributing to oral malodour by producing methanethiol, the VSC associated with periodontitis, and by influencing the tongue microbial composition and metabolism.

## Oral malodour Research Will Never Die

**Prof. Mel Rosenberg**

Tel Aviv University

As long as people breathe our research field will never die. There is so much yet to be investigated and developed. We need better techniques for measuring and classifying bad breath, advanced understanding of the underlying causes, the microbial and molecular mechanisms, helping individual patients, and coming up with novel treatments. During my career I have had the opportunity of working with remarkable individuals who are no longer with us, and yet their contributions to this field will remain forever. In my presentation, I will discuss the impact of several of these researchers, including Joseph Tonzetich, Israel Kleinberg, Walter Loesche and Anton Amman, as well as other researchers throughout the ages whose contributions and insight continue to illuminate our path forward.

## Exhaled Breath Real-Time Analysis and its Potential Application in Halitosis Research

Tanja Zivkovic Semren, Paul Alain Singh Kalra, Filippo Zanetti, Nikolai V. Ivanov, Magalie Ronzeau, **Philippe A. Guy**

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

**Introduction:** Exhaled breath is considered a useful biological matrix due to the presence of volatile organic compounds that provide valuable information about biochemical processes in the body, its non-invasive collection, and unlimited availability. Since human actions prior breath analysis can alter the compounds detected in exhaled breath, it is essential to define a standardized protocol for breath sample collection. Real-time analysis of exhaled breath has recently gained interest due to the development of advanced analytical techniques reinforcing its application in research of various diseases.

**Material and methods:** The real-time analytical technique of secondary electrospray ionization (SESI) was coupled with high-resolution mass spectrometry (HRMS), SESI-HRMS, to analyse exhaled breath samples and other aerosols.

**Results:** SESI-HRMS was successfully employed for real-time targeted **[1]** and untargeted analyses of exhaled breath. Our results showed that beverage intake or `brushing teeth impacted the chemical composition of exhaled breath. Smoking conventional cigarettes also affects the composition of the volatile organic compounds present in exhaled breath and is distinct in comparison to the use of alternative heated tobacco products.

**Conclusions:** These results provide insight into the variation in exhaled breath chemical composition and confirmed the potential of SESI-HRMS technology in this field. They demonstrate that this approach can be applied to elucidate the potential link between volatile compounds in exhaled breath and certain diseases and health conditions. Halitosis is a health condition characterized by an unpleasant odour in exhaled breath. Advanced breath analysis techniques and multi-omics approaches can be used to understand the interactions between oral bacterial species and their roles in halitosis both *in vitro* and *in vivo* [2].

[1] Application of secondary electrospray ionization coupled with high-resolution mass spectrometry in chemical characterization of thermally generated aerosols. T. Zivkovic Semren. S. Majeed, M. Fatarova, C. Laszlo, C. Pak, S. Steiner, G. Vidal-de-Miguel, A. Kuczaj, A. Mazurov, M.C. Peitsch, N.V. Ivanov, J. Hoeng, and P.A. Guy. *J. Am. Soc. Mass Spectrom.* **2022**, 33, 2147−2155, DOI: 10.1021/jasms.2c00222

[2] A literature review and framework proposal for halitosis assessment in cigarette smokers and alternative nicotine-delivery products users. F. Zanetti, T. Zivkovic Semren, J.N.D. Battey, P.A. Guy, N.V. Ivanov, A. van der Plas, and J. Hoeng. *Front. Oral Health* **2021**, 2, 777442, DOI: 10.3389/froh.2021.777442.

## Investigating the Headspace of Biofilms with an Optical Nose to Pave the Way for Point-of-Care Diagnostic

**Romaine Dubreuil**

Aryballe, Grenoble, France

Biofilms, complex microbial communities embedded in a self-produced extracellular matrix, pose significant challenges in various fields, including healthcare and environmental monitoring. Traditional point-of-care diagnostic methods often fail to accurately detect and characterize biofilm-related infections, emphasizing the need for innovative approaches. In this study, we propose the utilization of an optical nose to investigate the headspace of biofilms, aiming to revolutionize point-of-care diagnostics.

The headspace above a biofilm contains volatile organic compounds (VOCs) released by the microbial community, offering a potential non-invasive diagnostic avenue. The optical nose developed by Aryballe (called a Neose), inspired by the mammalian olfactory system, can selectively detect and differentiate VOC profiles, providing valuable insights into the biofilm composition and activity. On the other hand, the Selected-ion flow-tube mass spectrometry (SIFT-MS) is a very robust tool to detect and quantify in real time the composition of the headspace above biofilms. The drawback of this tool is the very high price.

In this work, we coupled these 2 instruments (SIFT-MS and Neose) in the same fluidic line in order to measure 3 types of biofilms: Escherichia Coli, Streptoccocus Aureus and Pseudomonas Aerginosa. A very good discrimination between the biofilms was achieved by both the optical nose and the SIFT which paves the way for extending the usage of this hand-held tool to other biofilms and strains. In the hand, the optical nose needs to be validated by lab devices such in order to bring new tool for clinicians in the future.

**Are bacteria good for your brain? Linking microbes, their metabolites, and the brain's defences.**

**Simon McArthur**

Centre for Oral Immunology & Regenerative Medicine, Faculty of Medicine & Dentistry, Queen Mary University of London, UK

That the microbial communities of the body can affect apparently wider physiological functions is now steadily becoming established. The impact of microbial actors upon the brain have received considerable attention, given that microbial influences have been identified on both cognitive functions, such as anxiety, stress, and memory, and in major neurological disorders including Alzheimer's disease, Parkinson's disease, and stroke. Despite their importance, exactly how these effects are brought is unclear but circulating microbe-derived metabolites have been implicated as major mediating factors.

Our group investigates the effects of different microbial metabolites upon the primary interface between the circulation and the brain, the blood-brain barrier (BBB), and how this can influence brain function. The BBB has a major defensive role, removing neural metabolic waste to the blood and preventing entry of potentially harmful agents into the delicate brain environment. Importantly, damage or disruption to BBB function occurs at the very earliest stages of neurological disease, including in Alzheimer's disease and in stroke, potentially contributing to disease pathogenesis. We hypothesise that microbial metabolites may be able to regulate these processes, influencing neurological disease risk and pathology.

We have investigated the effects of several classes of metabolites known to be produced by oral and other microbes upon the BBB, namely short chain fatty acids (SCFAs), methylamines and p-cresol conjugates. By combining transcriptomic analyses, cellular models of the BBB and murine studies of brain function and behaviour, we have identified the mechanisms whereby these molecules modify BBB integrity, effects that feed forward into changes in cognition and the response to neuroinflammation. This work demonstrates how microbe-derived molecules can directly influence brain health, and potentially, susceptibility to neurological disease.

## Oil Pulling Mouthwash as a Natural Alternative to Chemical Mouthwashes: An Exploratory Study

**Sushma Nachnani, Ph.D. Steve Lee, DDS**

Objective: This exploratory study aimed to assess the efficacy of an oral rinse product, specifically an oil pulling mouthwash, in controlling oral malodor as a natural alternative to chemical mouthwashes.

Methods: A randomized, double-blinded clinical trial was conducted with sixty adult volunteers who had oral malodor scores of at least 7.0 ± 0.5 on a hedonic scale. The participants were divided into two groups: Group B received the active oil pulling mouthwash, while Group A received a placebo product. The study duration was three weeks, consisting of three visits: baseline, 12-hour visit, and three-week visit. Organoleptic evaluation, oral examination, and demographic data collection were performed at each visit. Safety variables and product usage compliance were monitored. Efficacy was evaluated based on the comparison of organoleptic scores between the two groups.

Results: The oil pulling mouthwash contained various food grade oils such as Peppermint oil, Clove oil, Tea tree oil, Cardamom oil, Fennel oil, Vitamin E, D, K2, and stevia. Participants were instructed to use 10ml of mouthwash once daily for two minutes and to continue their normal oral hygiene practices. Statistical analysis included descriptive statistics, mean age differences, outcome comparisons, and paired/unpaired t-tests.

Conclusion: The study protocol was designed to evaluate the efficacy of oil pulling mouthwash as a natural alternative to chemical mouthwashes in controlling oral malodour. The findings from this exploratory study will provide insights into the potential benefits of oil pulling mouthwash in oral hygiene practices and may pave the way for further research in this area. Detailed data analysis will be included at the time of the presentation at the meeting.

**Why is smell important? How does it work? What happens when we lose our sense of smell? How can it be restored?**

**Thomas Hummel, M.D.**

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The sense of smell is important in our everyday life. We react e.g. B. to body odors of our fellow human beings - the nose determines who we "can smell". The perception of scents and thus also the perception of aromas when eating and drinking give us a large part of the quality of life. And the smell also influences our professional life: Some professional groups, such as the profession of perfumer, baker or cook, are absolutely dependent on an intact sense of smell. The sense of smell is essential for recognizing dangerous situations, such as recognizing spoiled food or the early detection of a smoldering fire.

In summary, one can say that smell is important in terms of interpersonal communication, as a warning sense and as a sense that is essential for many people to enjoy eating and drinking.

**Nitrate-enrichment reduces volatile sulfur compounds and activates the nitrate-nitric oxide pathway in salivary and anaerobic bacteria associated with human halitosis**

**M. Tian[2] Christine D. Wu[1], W. Li[1], and M. Dodds[2]**

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Dietary nitrate from foods, such as green, leafy vegetables and beets, can be metabolized to nitrite by nitrate reductase produced by anaerobic oral bacteria. Human gut bacteria convert nitrate to nitric oxide, which has been linked to reduction of blood pressure and maintaining cardiovascular health. Tongue microflora contains some of the most prevalent nitrate reducers in the oral cavity. Objectives: to investigate the effect of nitrate-containing compounds on VSC generation by human salivary bacteria and to investigate the effect of Magnolia Bark Extract (MBE) and flavor oils on nitrate reductase activity of salivary and halitosis-associated oral anaerobes. Methods: Stimulated whole saliva was collected from adult volunteers over a 10-minute period while chewing a 1g piece of unflavored unsweetened gum. Salivary bacteria and anaerobic oral bacteria commonly associated with human halitosis were mixed with cysteine (0.25%) and nitrate or nitrate-rich beet extract. Nitrate reductase activity was assayed using Griess reagent. VSC were detected using gas chromatograph equipped with a sulfur detector. Nitrate reductase activity in the presence of natural antimicrobial MBE and plant essential oils such as peppermint oil was examined. Results: Salivary bacteria, *Aggregatibacter actinomycetemcomitans, Actinomyces naeslundii* and *Veillonella atypica* were nitrate reductase producers. *Porphyromonas gingivalis, Fusobacterium nucleatum, Enterococcus faecalis* and *Streptococcus mutans* did not contribute significantly to salivary nitrite generation. In the presence of nitrate, salivary bacteria utilized nitrate-nitrite pathway and significantly reduced production of VSC. Flavored oils and MBE, at concentrations incorporated into chewing gum and mints, did not significantly suppress nitrate reductase activity of test bacteria. Conclusions: Oral bacteria with a ready source of nitrate may downregulate enzymes associated with halitosis, promote the generation of nitric oxide and contribute to cardiovascular health. Dietary nitrates may provide breath freshening benefit. Flavor oils and MBE when incorporated into chewing gum and mints at levels added in the products did not suppress nitrate reductase activity of oral anaerobes and human salivary bacteria.

## To Sniff or Not to Sniff, That is the Question

**Saliha Saad[1] and Patricia Lenton, presenters and facilitators**

[1] Centre for Research in Biosciences, University of the West of England and [2] Health Care Compliance and Clinical Research, Health Care Business Group 3M Center, St. Paul, United States

Clinical investigations of halitosis (oral malodor) typically use both objective and subjective assessment methods to rate breath odor levels. Objective methods use analytical instruments to measure the principle components, namely volatile sulfur compounds. Objective methods are not typically considered to put the evaluators at risk of catching potential illnesses that study subjects may be infected with. Subjective, or sensory methods (organoleptic assessments) are considered by most investigators to be the gold standard breath odor assessment method because of the sophistication of the olfactory sense, which is one of our oldest senses; for mankind, smell is one of the most important ways of interaction with the environment. With sensory methods, breath odor evaluators sniff subjects' mouth air directly from the mouth at a distance of approximately 6-inches. For decades researchers have raised concerns about sensory assessments and evaluator safety.

Numerous surrogate methods have been proposed, e.g., wrist-lick test, spoon test, syringe collection test, etc.) to replace the direct sniffing technique. However, many investigations continued to utilize direct sensory methods in their research trials. That is, until the onset of the Covid-19 pandemic. Covid-19 has elevated existing concerns about the use of direct sniffing methods for evaluating breath odor.

The aim of this session is to leverage the expertise of the international conference attendees, most of whom have been actively involved in breath odor research for many years, and through discussion of scientifically based sensory assessment strategies, the hope is to reach consensus on guidelines for the subjective evaluation of breath odor when conducting clinical research trials.

## The interplay between periodontitis and chronic kidney disease

**Mike Curtis**

**Kings College London, UK**

Periodontal disease, a microbially driven chronic inflammatory disease of the periodontal tissues, is associated with a number of systemic conditions including: diabetes, cardiovascular disease, rheumatoid arthritis and chronic kidney disease (CKD). However, whether these associations reflect a causal relationship between oral disease and systemic conditions or alternatively the influence of shared host/environmental risk factors remains unresolved. In recent years our laboratory has examined this issue using rodent models of a number of systemic conditions to provide the basis for hypothesis driven studies in human populations. This presentation will present findings from our recent studies of the interplay between periodontal disease and CKD using rat and mouse models of ureamia, analyses of microbial dysbiosis by 16S community profiling and microbial transfer experiments using germ free mice.

## The Oral Microbiome Unmasked: Microbiome Sequencing as a Tool for Understanding Oral Health

**Jegor Oleinikov**

**BioMarker Technologies (BMK) GmbH**

The Oral Microbiome is a complex and diverse community of microorganisms that inhabit the human mouth. Recent advancements in microbiome sequencing technology have allowed scientists to uncover new insights into the role of these microbial communities in oral health and disease. This presentation will explore the use of microbiome sequencing as a tool for understanding the oral microbiome and its impact on human health. We will examine the current state of research in this area, types of sequencing techniques and methods from Amplicon sequencing to Metagenomics and other sequencing strategies and discuss the potential implications for future treatments and therapies.

The integration of transcriptomics techniques, such as mRNA sequencing, small RNA sequencing, long non-coding RNA sequencing, and circular RNA sequencing, with the analysis of the oral microbiome offers a powerful approach to gain comprehensive insights into the complex interactions and functional dynamics of oral microbiome. These techniques allow researchers to explore the gene expression patterns and regulatory networks within the oral microbiome, shedding light on the underlying molecular mechanisms of oral diseases.

By leveraging transcriptomics data, researchers can identify potential diagnostic biomarkers that can aid in the early detection of oral diseases. This early detection enables timely intervention, leading to more effective treatment outcomes. Moreover, the in-depth understanding of the oral microbiome provided by transcriptomics approaches facilitates the development of targeted therapies tailored to specific microbial communities or dysregulated pathways, paving the way for novel treatment strategies.

## The effect of viral filters on the volatile profile produced by bacterial biofilms

**Dr Keith Hewett**

**Oral Malodour Research Group, University of the West of England, Bristol.**

During the SARS-CoV-2 pandemic of 2020-2022 all scientific research involving sampling of systemic breath, or the oral cavity was suspended. Whilst the direct threat from this virus has receded, it is clear that this kind of sampling remains not without hazard. The purpose of this study was to ascertain whether a filter could be used that removed potentially infective particulate matter from a sample, yet left volatiles unaffected. Volatiles produced by facultative anaerobic and anaerobic organisms in vitro and from the oral cavity were analysed by SIFT-MS with and without filters. Differences in the volatile profiles were statistically insignificant ($p>0.1$) in all experiments.

# Poster Abstracts

**Machine learning models to identify breath quality and oral microbiome changes**

**Authors: Jinwoong Nam, Lyndsay Schaeffer, Lauren O'Connell, Keith Carroll, Mark Vandeven, and Saliha Saad**

ABSTRACT: Data from a previously conducted clinical study examining the breath efficacy of a Dual Zinc plus Arginine dentifrice was used to explore the relationships between odor judge scores, SIFT-MS analysis of volatile metabolites, and the oral microbiome. Methods: We analyzed data from this study to identify changes in volatile metabolites and look at how those changes relate to breath quality and microbiome composition. In this data, the intensity of oral malodors was scored on a 0 to 5 scale (organoleptic score) by human judges for 480 samples. Also, 11 volatile organic compounds (VOCs) per sample were measured from mouth air by SIFT-MS. Lastly, the oral microbiome environment was investigated by 16S rRNA gene amplicon sequencing. For the analysis of data, we classified the high and low-breath quality groups based on the organoleptic scores. Then, various machine learning classifications and differential expression analyses were applied with the measured volatile compounds and identified salivary microorganisms. Results: The effect of dentifrice on breath quality was previously reported. In the present analysis, we were able to predict breath quality with an accuracy of 80% from VOCs measured by SIFT-MS. While hydrogen sulfide and methanethiol were two major contributors, other components such as acetic acid, propanoic acid, and trimethylamine played a role in breath quality. For bacteria, the relative abundances of Stomatobaculum, Peptostreptococcaceae, and Saccharibacteria were higher in the bad breath quality group whereas the relative abundances of Rothia, and Streptococcus were higher in the good breath quality group. Conclusion: Machine learning along with SIFT-MS and gene sequencing analysis helps identify relationships between breath components, oral microbiome environment, and breath quality, which is valuable information to further studies of oral malodor. It is also useful for the predictive analysis of the breath quality of individuals, preventing the risks inherent in measurements by human judges.

ABSTRACT SUMMARY The relationships between breath components, oral microbiome, and breath quality are investigated with machine learning.

KEYWORDS SIFT-MS, oral microbiome, oral malodor, machine learning.

**Efficacy of Transatak™ Malodor Control Technology in Different Product Formats and Flavors systems for Oral Malodor Relief**

**Javier Cuadros[1*], Steve Pringle[1], Matthias Pies[1], John Zanone[1], Christine Cahen[2]**
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Introduction: Bad breath is a common issue that affects individuals worldwide. Despite the many products available in the market that claim to address this issue, consumers often experience temporary relief with malodor often returning at previous levels. Various product formats, such as mint tablets, breath films, hard candy, and toothpaste, have been developed to offer different modes of delivery and formulation options.

Objectives: The objective of this study is to evaluate and compare the effectiveness of different product formats containing the Transatak® Malodor control technology, in reducing oral malodor, and compare them to control malodor reference, unflavored product base, and/or market samples.

Materials & Methods: Takasago has developed various Transatak® flavor systems that incorporate patented ingredients designed to react with and eliminate oral malodor components such as bacterial-generated Methyl Mercaptan. These Transatak® malodor control flavor systems have been formulated into several products, including an Active Charcoal toothpaste, Fresh Mint & Strong Mint tablets, and Spearmint hard candy samples. To evaluate the effectiveness of these products in reducing malodor, Takasago's sensory test methodology was used, which involved trained employee panelists who assessed the malodor of glass jars containing the tested products with water and the Methyl Mercaptan malodor reference on a 10-point malodor intensity scale (0-9).

Results: Takasago's proprietary Transatak™ flavor systems significantly outperformed control flavors in reducing malodor across various products, according to the sensory test data. The technology was effective in eliminating oral malodor components, including bacterial-generated Methyl Mercaptan, and was even shown to perform better than market products.

Conclusion: Overall, the sensory test suggests that Transatak™ flavor systems are an effective solution for reducing oral malodor components, including Methyl Mercaptan. Its efficacy was demonstrated across various products, including toothpaste, Mint tablets, Oral films, and Hard candy, and was even more effective than market products. Therefore, the sensory test method appears to be a valid and reliable tool for manual malodor evaluation of different products containing Transatak™ technology. Takasago International Corporation April 2023.

## The Potential of Faecalibacterium prausnitzii as a Next Generation Probiotic for Periodontal Disease

**Rachel Jones and Saliha Saad**

Periodontal disease (PD) is estimated to affect 796 million people globally. Those suffering from disorders including Diabetes Mellitus, have increased risk of PD. Biofilms (mixed bacterial communities) are integral in the mouth. However, when pathogenic bacteria (Gram-negatives) concentrations heighten in the oral cavity, dysbiosis can occur. Most healthy individuals combat this with an immune response, but those with underlying disorders experience prolonged inflammation, and several complications including tooth loss. Current treatments include invasive mechanical debridement, so antibiotics are becoming increasingly favoured. Probiotics are gaining traction as a new alternative, with halitosis, PD and dental caries all having probiotic treatments, usually based on Lactobacillus spp.

This research aims to determine if i) Faecalibacterium prausnitzii, a commensal bacterium found within the gut, has probiotic potential as a PD treatment, ii) to elucidate its mechanism of action and iii) to compare its efficacy against existing probiotics.

Selected Ion Flow Tube-Mass Spectrometry will measure the changes in volatile/ sulphur compounds (VC/VSCs) released from PD implicated bacterial co-culture biofilms. Once established, biofilms will be treated with F. prausnitzii, and measured for change in VC/VSC levels and compared with controls (antibiotics, marketed probiotics). Bacteria concentration levels will be monitored to evaluate F. prausnitzii's impact. If successful, tongue scrapes from consenting volunteers will be used to inoculate the biofilm matrix, replicating real oral microbiomes, in addition to elucidating the mechanism/s of action. Safety assessments to determine toxic/adhesive traits will be completed in cell culture. These assessments will enable forecasting of F. prausnitzii's potential in clinical PD context.

## Biofilms and Volatile Detection: Challenges, Future Direction, and Sensing Technologies

**HRM Modha, DM Reynolds, F Barnes, S Onwugbenu, RMS Thorn**

Bacterial life on Earth mostly exists in a biofilm state, playing an important role in ecological cycles. However, the negative impacts of biofilm infections lead to food spoilage and wastage in agriculture and increased suffering and death rates in healthcare. Low molecular weight volatile compounds released by bacteria provide a promising avenue for early detection to tackle these challenges. Being able to identify the unique bacterial volatile profiles/fingerprints could form the foundation for rapid detection and identification. Current accurate volatile detection technologies i.e., Gas Chromatography-Mass Spectrometry and Selective Ion Flow Tube Mass Spectrometry are widely used but difficult to implement for real-world applications. Rapid sensing technologies i.e., E-Noses and Metal Oxide Sensors have paved the way for portability but lack real-time detection of multiple volatiles and data analysis to enable bacterial identification. Recent advances in graphene-based sensing technologies have provided a platform for a prototype volatile sensor (developed by Altered Carbon Ltd.) that this study aims to investigate in addition to the challenges around bacterial volatile detection and analysis.

Efficient and accurate volatile sampling for laboratory testing remains a barrier to advances in bacterial volatile detection. Consequently, we have developed a system which allows closed-system volatile sampling with minimal losses. The system enables; (1) easy introduction of samples, (2) introduction of known gases/volatiles for interaction studies, (3) multiple sampling chambers, (4) adaptability to test multiple detection systems simultaneously, and (5) provides minimal time delays between detectors for robust data comparison. This successfully optimised system provides a platform for future research on bacterial volatiles and sensing technologies.