BREATH® BIOPSY VOC Atlas

INTRODUCING THE BREATH BIOPSY® VOC ATLAS: A LIST OF MICROBIOME-ASSOCIATED VOCS FOR BREATH-BASED BIOMARKER DISCOVERY

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Aims

- Utilize matched breath and blank samples to characterize microbiome-associated volatile organic compounds (VOCs) in human breath.
- Define "on-breath" microbiome-associated VOCs present above background levels using multiple comparisons.
- Establish ranges for microbiome-associated, on-breath VOCs observed in nominally healthy and disease populations.

1. Background and Objectives

Volatile organic compound (VOC) biomarkers in exhaled breath offer a promising solution to safe, non-invasive diagnostics in multiple disease settings. Some of these VOCs are linked to the gastrointestinal (GI) microbiome and could provide insight into disease development.

Many breath-based studies have been performed in several disease conditions, but there are few validated breath-based biomarkers currently in clinical use[1]. The complex and unknown composition of the breath matrix makes identification and validation of on-breath VOC biomarkers challenging. The Atlas study was designed to provide a comprehensive catalog of VOCs identified on exhaled human breath, the Breath Biopsy VOC Atlas, to support development of breath VOC biomarkers and diagnostics. Here, we highlight microbiome-associated VOCs, identified from two cohorts, contained in this ever-expanding VOC Atlas.

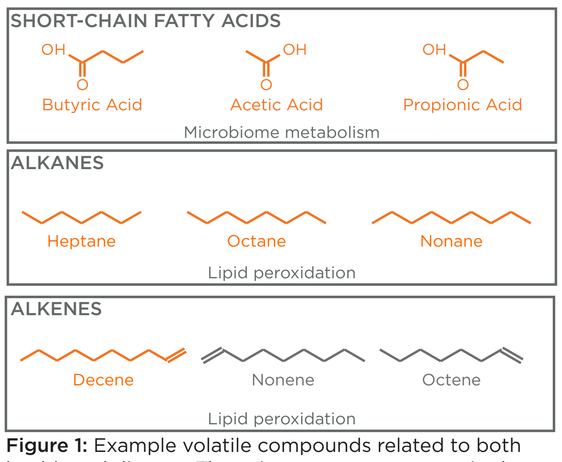


Figure 1: Example volatile compounds related to both health and disease. Those in orange are present in the VOC Atlas.

2. Method

Study Cohort and Breath Sample Collection:
In total, 170 adult volunteers were enrolled between 2022 and 2023 in Cambridge, UK. All volunteers provided written informed consent and breath samples were collected according to IRB-approved protocols at Owlstone Medical's Breath Biopsy Laboratory (Cambridge, UK).

For project 1, we collected 94 breath samples from heterogenous volunteers and 94 blank samples. For project 2, we collected a total of 76 breath samples from healthy/prediabetic/type 2 diabetic volunteers and 66 blank samples. All breath samples were collected using Owlstone Medical's Breath Biopsy OMNI sample collection and analysis platform. All equipment blank samples were generated by collecting room air.

Breath Sample Analysis:
Samples were analyzed using thermal desorption - gas chromatography - mass spectrometry (TD-GC-MS) (Thermo Scientific, Waltham, MA, US). On-Breath Calculations: Molecular features were determined to be originating from breath or "on-breath" using three criteria. 1) Sample signal of at least 50% of breath samples > Mean + 3 standards deviations of background. 2) Paired t-test with p values <0.05 and fold change >2 and time-matched blank samples, with a mean fold change > 2. 3) ROC-AUC > 0.8 and a positive mean fold change between breath and blank. VOCs confirmed to be on-breath were identified using purified chemical standards.

Table 1: Project 1 study cohort displayed by age and sex.

Age Group (Yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

Table 2: Project 2 study cohort displayed by health status.

Inclusion Criteria	Variable	Healthy Control	Prediabetic	Diabetic
	N	29	20	27
30-65	Mean Age (SD)	46.7 (9.9)	48.2 (8.9)	50.5 (7.6)
>25	Mean BMI (SD)	29.7 (4.2)	33.5 (6.6)	36.8 (7.7)
	Sex (Female), n	15	13	14
Fast For > 2h	Mean Hrs Since Last Meal (SD)	5.9 (4.9)	8.3 (5.5)	7.4 (5.1)
	Mean Hrs Since Last Drink (SD)	6.5 (6.3)	9.2 (5.6)	6.4 (5.3)
	Smoking, n	2	0	6
Glucose Test Onsite	Mean Glucose Level (SD)	5.2 (0.5)	6.1 (0.8)	7.8 (1.8)

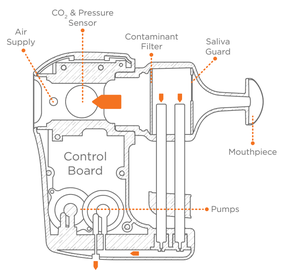
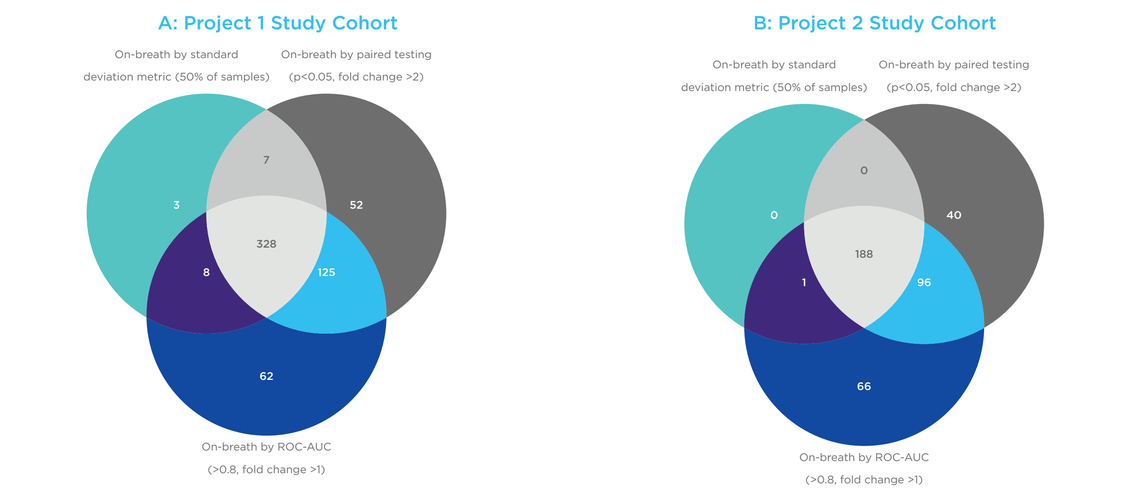


Figure 2: ReCIVA® Breath Sampler.

3. Results and Discussion



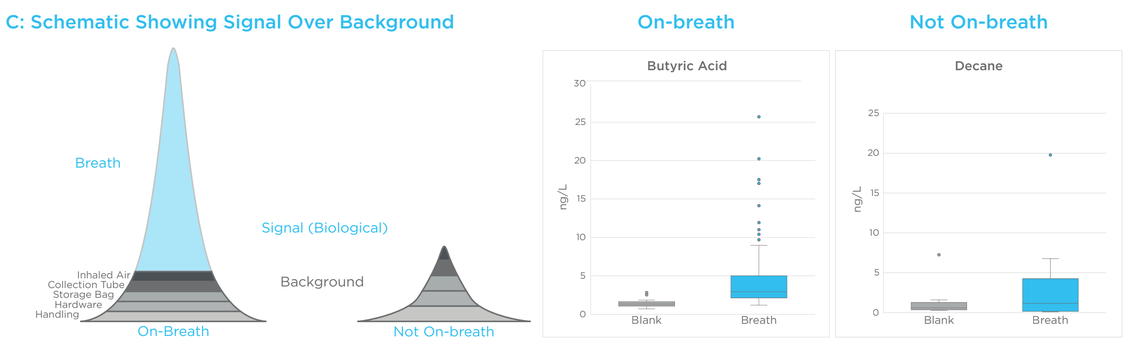


Figure 3: On-breath features (n) by each criterion. A) For cohort 1, 1471 total features were identified in breath samples, of these 328 are on-breath by all 3 criteria. B) For cohort 2, 1267 total features were identified in breath samples, of these 188 are on-breath by all 3 criteria.

C) An on-breath compound must be distinguishable from background noise: (Individual) area under peak ≥ mean background + 3 x standard deviation background (right).

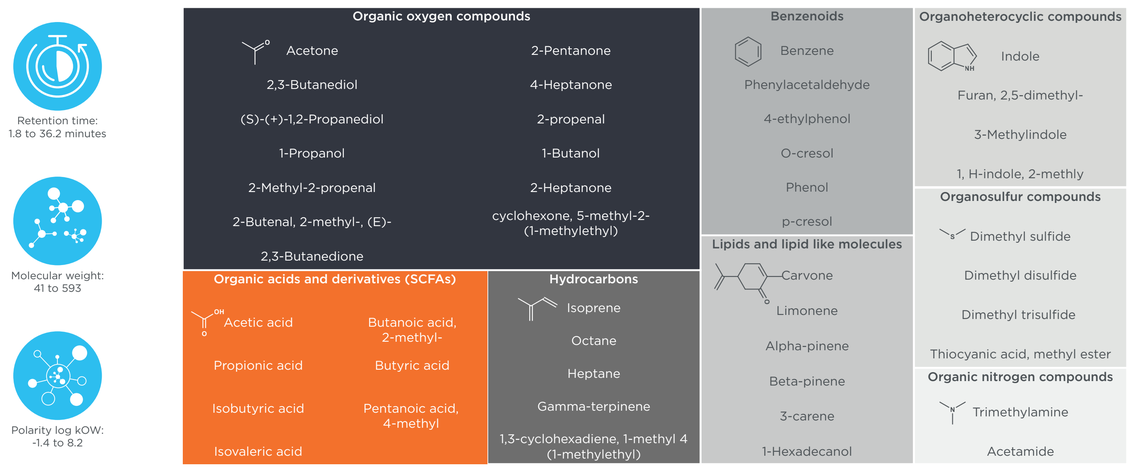


Figure 4: Summary of microbiome-associated Breath Biopsy Atlas contents. Currently, >180 identified VOCs, with 47 being microbiome-associated, have been identified using comparisons to purified chemical standard. On-breath VOCs reflect multiple chemical classes (right) and chromatographic properties (left). Categories will expand as additional VOCs are added to the Atlas.

The Microbiome

This Atlas currently contains >180 identified VOCs, with 47 being microbiome-associated, including short-chain fatty acids (SCFAs). In both populations studied here using the Breath Biopsy OMNI platform, SCFAs were reliably detected: 1.20 - 25.69 ng/L butyric acid (median= 2.92), 0.01 - 81.47 ng/L acetic acid (median= 11.73) and 0.01 - 46.20 ng/L propanoic acid (median= 1.2).

Dietary fibers, which cannot be processed by humans, are fermented by microbes in the intestines, resulting in the production of VOCs such SCFAs. These VOCs have roles in several signaling contexts including the central nervous system, immunity, and inflammation. A study from Smolinska et al. demonstrated the connection between exhaled breath VOCs and intestinal microbiota in subjects with Crohn's disease (CD)[2]. Samples were collected and analyzed in the same subjects during both active symptom flares and during remission. Acetate and propionate (both in the Atlas) correlated significantly with *Bifidobacteria* and several other microbes in the Firmicutes phylum in both disease states. Moreover, the microbial strains and the relative abundances of SCFAs both decreased in the active disease state[2].

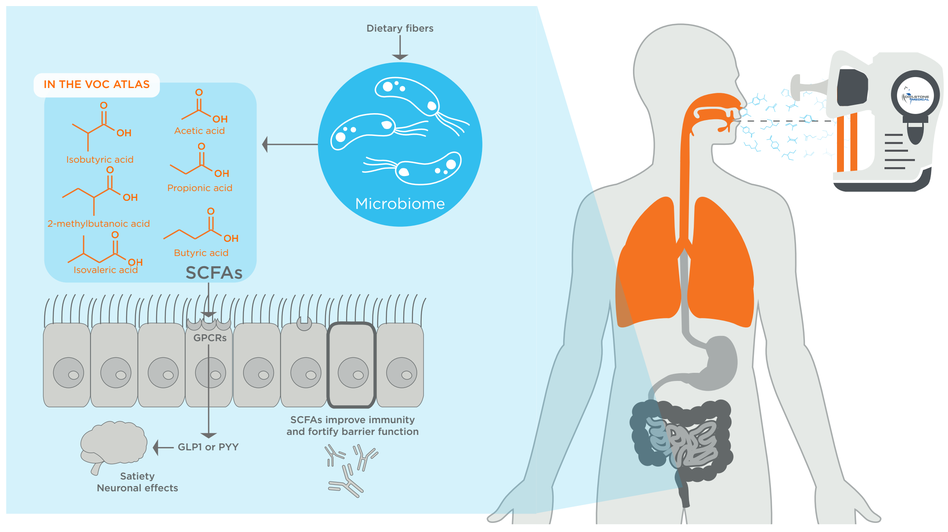


Figure 5: VOCs in the Breath Biopsy Atlas are potentially produced by microbiome activities thus on-breath VOCs could be a non-invasive source of microbiome biomarkers.

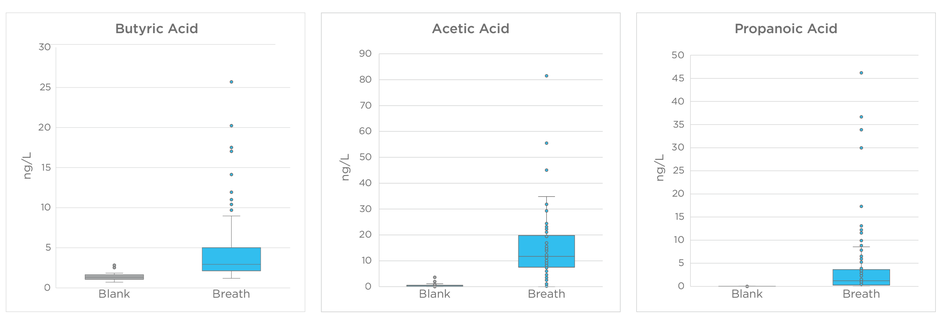


Figure 6: Butyric, acetic and propanoic acids are SCFAs that are present the in Breath Biopsy VOC Atlas. They can be detected at levels significantly above background signals in exhaled breath using Owlstone's technology. These SCFAs are notable products of microbiome metabolism that could inform on microbiome activity.

Other microbiome-associated VOCs

Other interesting microbiome-associated VOCs present in the Breath Biopsy Atlas are related to inflammation such as indole, trimethylamine and isoprene. Indoles have been linked to maintaining the biological barrier of the human intestine[3]. Trimethylamine (TMA) is linked to heart disease via trimethylamine N-Oxide (TMAO). TMAO can promote release of inflammatory cytokines, enhance the monocyte adhesion to the endothelial cells, and promote the oxidative stress[4], and isoprene-derived aerosols have been found to alter inflammatory/oxidative stress genes[5]. These three VOCs are present in the Breath Biopsy Atlas.

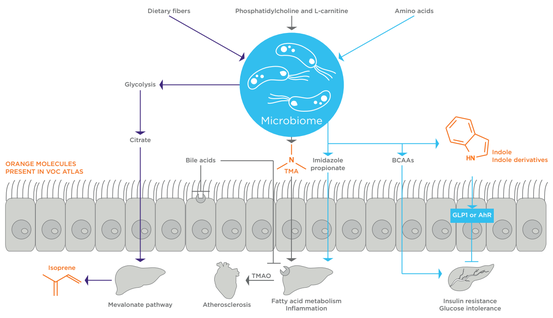


Figure 7: VOCs in the Breath Biopsy Atlas are potentially associated with the activity of the fatty acid oxidation pathway. Additional experimentation will elucidate mechanistic associations between on-breath VOCs.

4. Conclusions

The Breath Biopsy VOC Atlas, a growing list of VOCs that exist on-breath, is a tool to facilitate microbiome VOC biomarker discovery. It aims to provide a comprehensive suite of on-breath VOCs and reference ranges in a diverse healthy population that can be used as a comparison in multiple disease contexts and to characterize relationships between physiology and breath VOCs.

The ability to reliably detect microbiome-associated VOCs, paired with a curated repository of VOCs found on-breath could transform microbiome research. The Breath Biopsy VOC Atlas could aid in biomarker discovery by providing normal ranges for key compounds in healthy subjects that can later be compared to levels in disease cohorts. Furthermore, alongside other tools such as metagenomics and inflammation profiling, the Atlas could provide a more holistic view of the role of the gastrointestinal microbiome in disease development.

References

1. Haworth, J. J., et al. (2022). "Breathing new life into clinical testing and diagnostics: perspectives on volatile biomarkers from breath." Crit Rev Clin Lab Sci 59(5): 353-372. DOI: 10.1080/10408363.2022.2038075)
2. Smolinska, A., et al. (2018). "Volatile metabolites in breath strongly correlate with gut microbiome in CD patients." Anal Chim Acta.1025: p. 1-11.DOI:10.1016/j.aca.2018.03.046
3. Ye X et al. (2022). "Dual Role of Indoles Derived From Intestinal Microbiota on Human Health." Front Immunol. 17;13:903526. doi:10.3389/fimmu.2022.903526. PMID: 35784338; PMCID: PMC9248744.
4. Liu, Y et al. (2020). "Trimethylamine N-Oxide Generated by the Gut Microbiota Is Associated with Vascular Inflammation: New Insights into Atherosclerosis." Mediators Inflamm. Article ID 4634172, 15 pages. https://doi.org/10.1155/2020/4634172
5. Eaves, LA et al.(2020). "Isoprene-Derived Secondary Organic Aerosol Induces the Expression of MicroRNAs Associated with Inflammatory/Oxidative Stress Response in Lung Cells." Chem Res Toxicol. 33 (2), 381-387 DOI: 10.1021/acs.chemrestox.9b00322