BREATH® BIOPSY

VOC Atlas

Introducing Breath Biopsy® VOC Atlas: A List of Known Breath VOCS for Breath-based Biomarker Discovery

Ace Hatch, PhD, Hsuan (Tina) Chou, PhD, Owen Birch, Oliver Dean, Wisenave Arulvasan, Simon Coplowe, PhD, Julia Greenwood, Lara Pocock, and Billy Boyle

Owlstone Medical Ltd., Cambridge, UK, *email: breathbiopsy@owlstone.co.uk

Aims

- Utilize matched breath and blank samples to characterize the volatile organic compound (VOC) contents of human breath
- Define a set of "on breath" VOCs present above background levels using multiple comparisons of signal strength between breath and blank samples
- Establish ranges of on breath VOCs observed in a healthy population and begin investigating potentially relevant biological contexts

1. Background and Objectives

Volatile organic compound (VOC) biomarkers in exhaled breath offer a promising route to safe, non-invasive diagnostics in multiple disease settings. Many breath-based studies have been carried out in a range of conditions, including respiratory diseases, 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 a significant challenge.

The Atlas study was designed to provide a comprehensive catalogue of VOCs identified on exhaled human breath, the Breath Biopsy VOC Atlas, to support development of breath VOC biomarkers and diagnostics. Here we present the study cohort, breath sampling procedure, and analysis leading to the Breath Biopsy VOC Atlas.

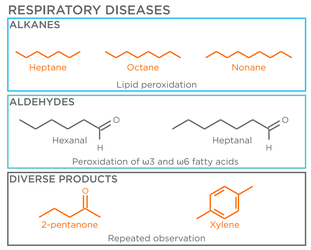


Figure 1: Example volatile compounds associated with respiratory inflammation. Those in orange are present in the VOC Atlas.

2. Methods

Study Cohort: 94 adult volunteers were enrolled in January/February 2022 in Cambridge, UK. All volunteers provided written informed consent and breath samples were collected according to an IRB-approved protocol at Owlstone Medical's Breath Biopsy Laboratory (Cambridge, UK).

Breath Sample Collection: Volunteers provided breath samples using Owlstone Medical's Breath Biopsy OMNI sample collection and analysis platform. A paired equipment blank was generated by sampling room air immediately prior to each breath sample collection. The ReCIVA® Breath Sampler is shown in Figure 2. The final analysis cohort consists of 90 volunteer breath samples and paired equipment blanks (Table 1).

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 > 3 standard deviations higher than background 2) Paired t-test p-value <0.05 and fold difference >2 between breath and paired blank 3) receiver operating characteristic area under the curve (ROC-AUC) >0.8. VOCs confirmed to be on breath were identified using purified chemical standards.

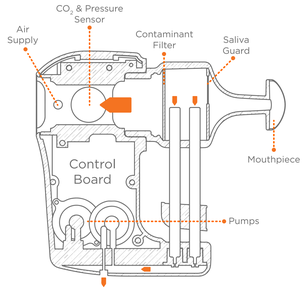


Figure 2: ReCIVA® Breath Sampler.

Study Cohort

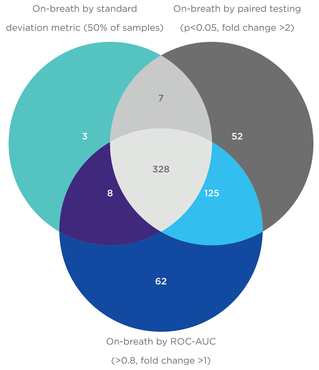
Age Group (Yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

Table 1: Study cohort displayed by age and sex.

References

1. Haworth, J. J., et al. (2022). "Breathing new life into clinical testing and diagnostics: perspectives on volatile biomarkers from breath." Critical Reviews in Clinical Laboratory Sciences 59(5): 353-372. DOI: 10.1080/10408363.2022.2038075)
2. Kelley, E., et al., Breath Biopsy® of Elite Runners Engaging in Exhaustive Exercise. Poster presented at Breath Biopsy Conference; Nov 10, 2020; Online.
3. Smolinska, A., et al., Volatile metabolites in breath strongly correlate with gut microbiome in CD patients. Anal Chim Acta, 2018. 1025: p. 1-11. DOI: 10.1016/j.aca.2018.03.046

3. Results and Discussion



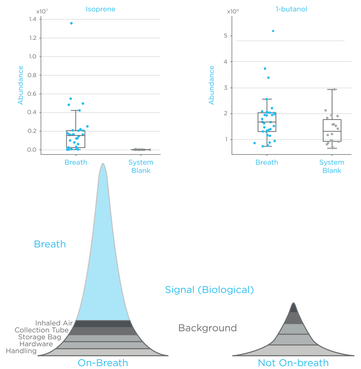


Figure 3: On-breath features (n) by each criterion. 1471 total features were identified in breath samples, of these 328 are on breath by all 3 criteria (left). An on-breath compound must be distinguishable from background noise: *(Individual) area under peak ≥ mean$_{background}$ + 3 x standard deviation$_{background}$* (right).

Compound ID Workflow

Candidate compound selection & preparation

↓

TD-GC-MS Orbitrap Thermo Scientific™ Q-exactive analysis + export

↓

Library spectra addition

↓

Manual feature matching

↓

ID confirmation

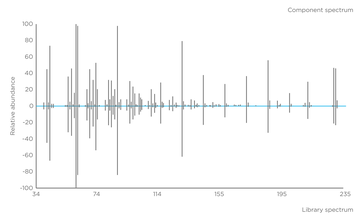


Figure 4: VOC Identification process workflow (left). Example comparison of standard and on-breath feature spectra for VOC identification (right).

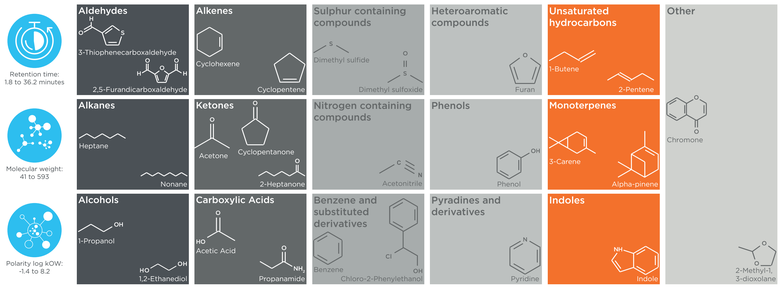


Figure 5: Summary of Breath Biopsy Atlas contents. Currently, 150+ on-breath VOCs have been identified using comparisons to purified chemical standards. On-breath VOCs reflect multiple chemical classes (right) and chromatographic properties (left). Categories will expand as additional VOCs are added to the Atlas.

Fatty Acid Oxidation

Several of the VOCs in Atlas belong to the fatty acid beta-oxidation pathway. Fatty acids are an important energy source, and their abundances fluctuate with circadian rhythms and energy demands. Through the process of fatty acid oxidation, multiple acetyl coA molecules are generated by repeatedly removing two-carbon groups from larger acyl CoA molecules until the precursor is completely oxidized. This process is highly efficient and fatty acids that enter this process are generally completely metabolized. However, volatile ketones such as 2-pentanone may arise through incomplete oxidation. Acetyl CoA itself can give rise to several volatile molecules, such as acetic acid through hydrolysis, acetaldehyde through acetaldehyde dehydrogenase, and acetone through carboxylation. in a previous collaboration with Mayo Clinic, we compared breath VOC profile changes pre- and post-race in ultra marathon runners. Several Atlas VOCs potentially generated by the fatty acid oxidation pathway (acetone, 2-heptanone and 2-pentanone) were found increased in post-race runners, implying the usage of fatty acid oxidation as an energy source[2].

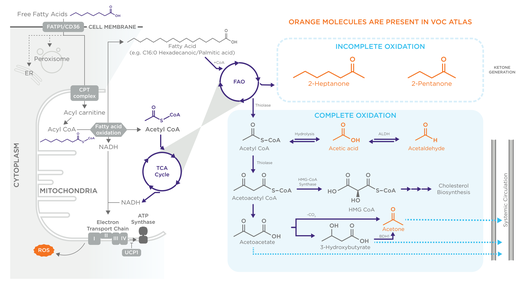


Figure 6: VOCs in the Breath Biopsy Atlas are potentially associated with the activity of the fatty acid oxidation pathway. Additional experimentation will elucidate mechanistic associations between on-breath VOCs.

The Microbiome

VOCs can also be produced by the gut microbiome. Dietary fibers, which cannot be processed by humans, are fermented by microbes in the intestines, resulting in the production of VOCs such as short chain fatty acids (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) 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[3].

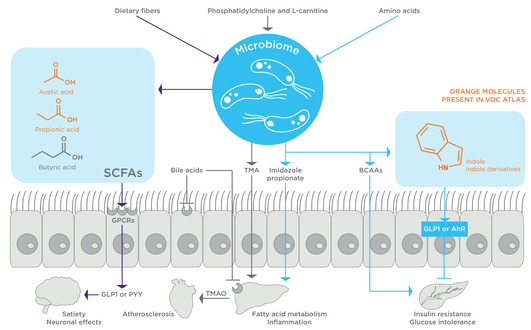


Figure 7: VOCs in the Breath Biopsy Atlas are also potentially produced by activities of the microbiome. On-breath VOCs could be a non-invasive source of microbiome biomarkers.

4. Conclusions

The Breath Biopsy VOC Atlas is an ever-growing list of VOCs that exist on-breath, identified using purified chemical standards, and differentiated from background contamination using system blanks generated at the time of breath sample collection. This tool will facilitate future VOC biomarker discovery efforts by providing 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.