Breath Biopsy® OMNI: Advanced Global Breath VOC Analysis

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1. Background and Objectives

There is a critical need for better ways to detect, monitor and treat diseases. Early detection and precision medicine have emerged as areas that have great potential to save lives and reduce costs by improving how we diagnose and treat illnesses.

Volatile organic compounds (VOCs) on breath have attracted growing interest as a promising biomarker source that may be relevant for a wide range of clinical applications. Over 1,000 different VOCs have been detected in human breath and these have both endogenous and exogenous origins (Figure 1).

Endogenous VOC sources incluce cell metabolism, inflammation, oxidative stress and pathogen metabolism. VOCs can be carried in the blood and exchange readily into air in the lungs. As such, biomarkers relevant to illness anywhere in the body could be detectable on breath.

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The most notable advantage of breath testing is that collection can be completely non-invasive, which makes it pain-free, easy to use and well tolerated by patients [1].

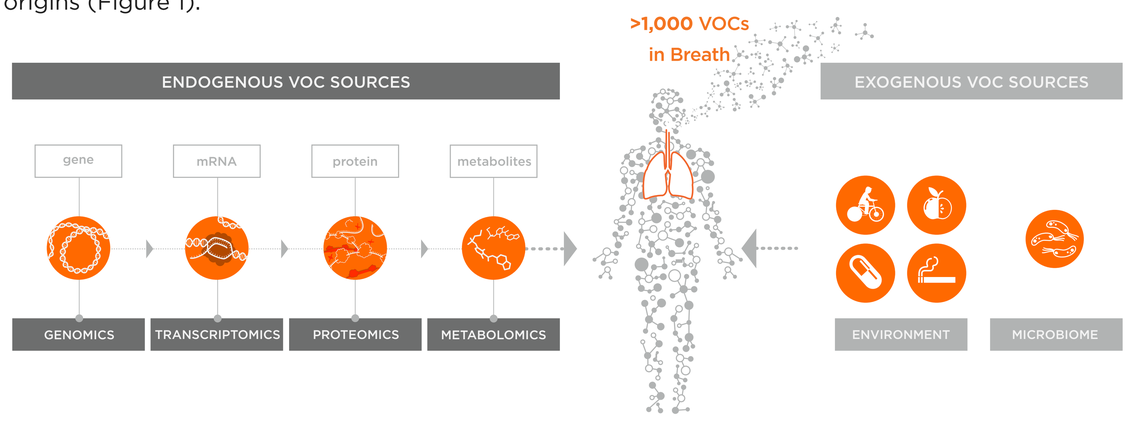

Figure 1. Volatile organic compounds (VOCs) from endogenous and exogenous sources can be detected on breath. VOCs produced by metabolic processes all over the body are carried to the lungs by the blood.

Progress Toward Breath Biomarkers

Studies have been published investigating the potential of breath biomarkers in a range of contexts including cancers, respiratory diseases, liver diseases, neurological conditions, gastrointestinal disorders and many more. While the majority successfully report candidate biomarkers, few have progressed these beyond pilot studies.

The lack of evidence to support progressing biomarkers further is largely due the diversity of methods used and and variation in how results are reported. Making progress depends on finding an approach to breath analysis that produces consistent, reliable and reproducible results.

Accurate biomarker identification is critical for developing clinically viable breath tests. Biomarker identities make it possible to understand how they relate to disease processes, supporting their use in disease detection and monitoring. Notably, this is relevant when seeking regulatory approval.

Breath Biopsy OMNI provides an end-to-end pipeline for robust collection and global analysis of VOCs on breath (Figure 2). Based on high resolution accurate mass (HRAM) GC-MS technology, the system has detection capabilities across six orders of magnitude and sensitivity as low as parts per trillion.

Figure 2. Breath Biopsy OMNI is a complete, end-to-end solution for global breath VOC analysis. In addition to an award-winning collection system and HRAM GC-MS analysis capabilities we include expert support for study design and management, statistical analysis and biological interpretation. Plus, there's several reporting options for you to choose from.

2. Method

Breath is collected using ReCIVA® Breath Sampler, a unique standardized, reproducible breath sampling device. ReCIVA operates with CASPER® Portable Air Supply, which provides low background inhaled air, minimizing environmental impact and reducing sample variability caused by ambient VOCs.

ReCIVA captures exhaled VOCs onto adsorbent tubes, allowing easy storage and transport of samples for analysis. Samples are shipped to our Breath Biopsy Laboratory and mass detection is performed using a HRAM Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific)

Distinguishing Features of OMNI

Study design: Our clinical and translational scientists can help you to answer key research questions by assessing factors such as sample size, inclusion and exclusion criteria, control group election and potential confounders.

QUESTIONS TO ASK DURING STUDY DESIGN:
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Power calculations: Our biostatisticians provide insights to help estimate the statistical power and minimum sample sizes needed to help your study achieve desired clinical performance.

Sample collection: ReCIVA makes collection convenient and comfortable for most subjects, including those with breathing difficulties or reduced lung function. Our optimized sampling approach takes around 15 mins and samples the lower breath fraction. While these are our optimized settings, they can be adjusted for specific applications.

Blank samples: Collecting system blank samples helps to assess undesired VOC sources, while background samples (process blanks) help assess signal to background for detected VOCs.

Quality assurance: Sample integrity and resulting analysis is monitored and assessed at all stages to assure reliable results. This includes purging and reconditioning of sorbent tubes between uses, performance monitoring during sampling, use of internal standards, use of calibration curves, storage methods that minimize sample degradation, and quality control checks by our biostatisticians and translational biologists during analysis.

VOC Identification

Detected compounds can be assigned identities on the basis of matching to libraries of standard compounds. For some VOCs, mass spectra and retention times can be compared to the Breath Biopsy HRAM Library, which has been developed using the same analysis technology, so offers more reliable comparison to OMNI data.

Depending on the specifics of your study design, we can offer a range of reporting options for your results. This can vary from a normalized feature table to custom uni- and multivariate analysis with biological interpretations.

References

1. Holden KA, Ibrahim W, Salman D, et al. Use of the ReCIVA device in breath sampling of patients with acute breathlessness: a feasibility study. ERJ Open Res. 2020 Oct;6(4).
2. Ferrandino G, Orf I, Smith R, et al. Breath Biopsy Assessment of Liver Disease Using an Exogenous Volatile Organic Compound—Toward Improved Detection of Liver Impairment. Clinical and Translational Gastroenterology. 2020;11(9):e00239.

Key Points

- OMNI is the most advanced global breath VOC analysis service
- It is a complete solution for breath collection and analysis including expert study design and reporting, as well as collection and analysis technologies
- It can support research aiming to characterize disease endotypes and therapeutic responses for early detection, precision medicine and drug development
- Data are high confidence, reliable and reproducible

3. Results

OMNI enables reliable, broad-ranging investigation of exhaled breath VOCs, increasing the chances to find clinically valuable biomarkers. We aimed to maximize the number of detectable VOCs on breath and to minimize introduced variability.

On breath VOCs are defined with high confidence (99.7%) as achieving a signal measured on breath that is at least three standard deviations above the average abundance in blank samples.

To facilitate method development, we used breath or blank (BoB) studies, which compare breath samples to representative blanks in order to offer a standardized approach for breath research that can be used in conjunction with any breath collection and analysis workflow.

FIND OUT MORE ABOUT BoB STUDIES:
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Comparing breath vs. blanks allows the discrimination of on-breath VOCs from other VOCs that are likely to be contaminants ensuring that only relevant VOCs are included for detailed analysis.

In one instance, BoB analysis was applied on 57 breath samples, obtained from four different volunteers, with an equal number of representative blanks (Figure 3). The method detected a median of 1,454 VOCs per sample of which a median of 517 were shown to be on breath.

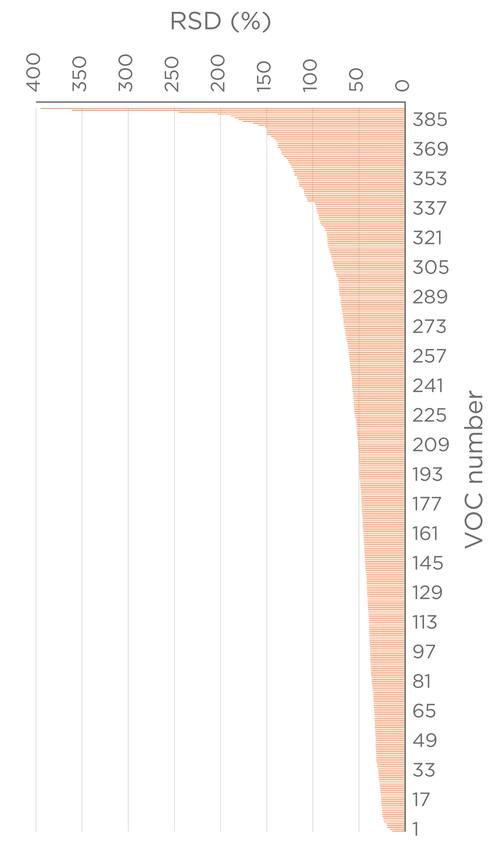

Figure 3. Breath Biopsy OMNI is optimized to provide consistent, reproducible results for breath analysis. In trials involving four healthy volunteers, the median intrasubject RSD for on breath VOCs was 26-36%. Across all samples, the median intersubject RSD was 49% across 392 VOCs.

4. Case Study

OMNI represents an evolution of Breath Biopsy, which is being used for a range of applications by researchers worldwide. One of our current projects aims to develop a breath test to detect non-alcoholic steatohepatitis (NASH) an early stage of chronic liver disease.

A pilot study looking at late stage liver disease (cirrhosis) found that limonene correlated with disease severity and blood-based biomarkers of liver function [2].

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We have since extended this work by performing global VOC analysis of breath from 46 people with cirrhosis and 42 healthy controls. The study identified 29 VOCs that differed significantly between groups, of which 11 also correlated with blood biomarkers (Figure 4).

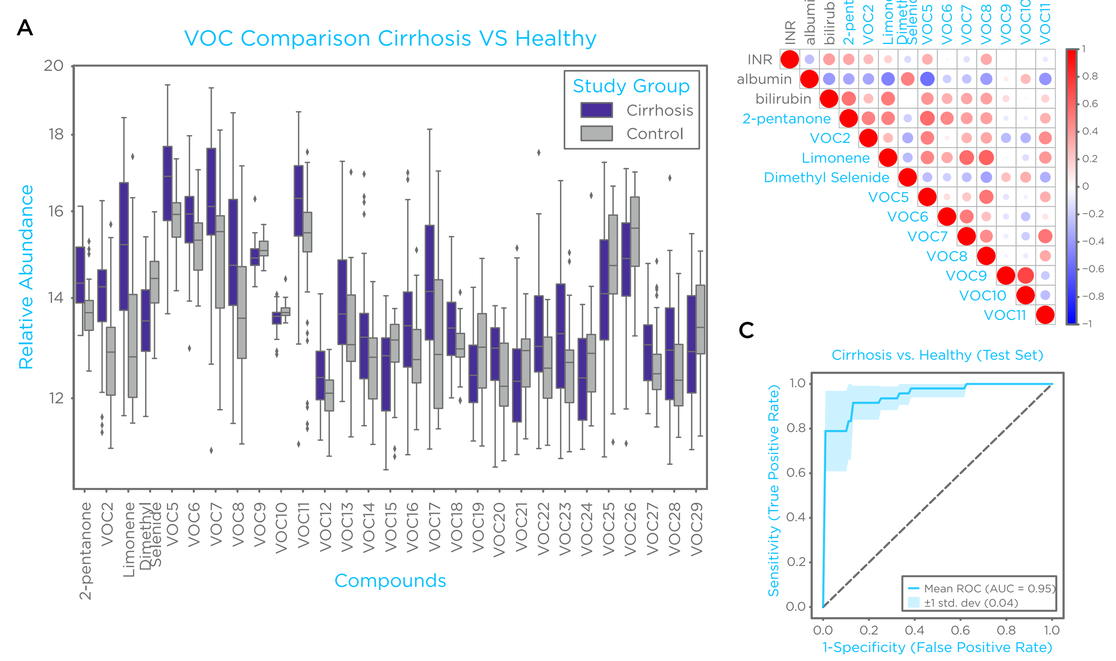

Figure 4. Breath Biopsy OMNI Global VOC Analysis of Cirrhosis vs. Healthy Controls. Paper in production. (A) 29 VOCs on breath identified as significantly different between cirrhosis and healthy controls including limonene and other previously reported compounds (2-pentanone and dimethyl selenide). (B) Correlations between blood biomarkers (INR, albumin and bilirubin) and 11 of the most significant identified VOCs. (C) Models built using measurements of all 29 VOCs applied to a test dataset resulted in a mean AUC of 0.95 with 83% true positive rate and just 7% false positives.

5. Conclusions

Development of breath tests has been limited by lack of standardized methods and reporting. This has resulted in many proposed biomarkers but few that have successfully advanced through validation.

OMNI has been developed as a global breath VOC analysis to address this need. Whatever application you are seeking biomarkers for, contact us about integrating Breath Biopsy OMNI into your work and to discuss your study design with our expert team.

For full details on Breath Biopsy OMNI, download our whitepaper: