Development of a new breath collection method for analyzing volatile organic compounds from intubated mouse models

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1. Introduction and Aims

Interstitial lung disease (ILD) is an umbrella term used for a large group of respiratory diseases that cause inflammation and scarring of the lungs. Idiopathic pulmonary fibrosis (IPF) is a form of ILD, where the lungs become scarred and fibrotic, meaning breathing becomes increasingly difficult. There is a significant delay in the diagnosis of IPF, which could be due to the fact that IPF is hard to diagnose as its symptoms are similar to other lung conditions such as chronic obstructive pulmonary disease (COPD). Early detection methods and more accurate biomarkers are needed to improve the treatment outcomes of IPF patients.

Exhaled breath contains hundreds of VOCs, including those originating from biological processes in the body, and therefore have the potential to serve as biomarkers for clinical applications. Breath is in direct contact with the respiratory tract, and so is especially enriched for VOCs originating from respiratory processes.

To reduce the variability and challenges associated with biomarker development, utilizing mice in controlled laboratory settings is necessary for expediting the identification and validation of breath biomarkers for clinical use.

Therefore, we collaborated with Boehringer Ingelheim to develop a reliable system for studying mouse breath in the laboratory, that can be tailored for respiratory disease research. The platform we provide enhances on-breath VOC signals by significantly reducing background noise. This will improve foundational studies of breath and expedite the translation of breath analysis to the clinic.

2. Methods

A total of 15 intubated C57BL/6JRj mice breath samples were collected using gold-standard respiratory mechanics equipment - a modified flexiVent® small animal ventilator (SCIREQ, Montreal, Quebec, Canada). An ambient filter and a flexiVent filter were both connected to the ventilator by flexible tubing and a sorbent tube for breath collection (Figure 1).

To compare mice and human breath profiles, a total of 13 healthy human volunteers were recruited. Each study participant provided a single breath sample collected using the ReCIVA® Breath Sampler.

For both mice and human samples, system blanks were collected to help distinguish breath from background compounds. All samples were analyzed using the Breath Biopsy® OMNI® standardized breath analysis method via thermal desorption gas chromatography and mass spectrometry (TD-GC-MS).

VOCs were identified through in-house HRAM and external NIST libraries, with compound IDs assigned following the Metabolomics Standard Initiative (MSI) standards.

Three metrics- standard deviation, paired t-tests and receiver operating characteristic area under the curve (ROC-AUC) were used in combination to determine VOCs 'on-breath' from background.

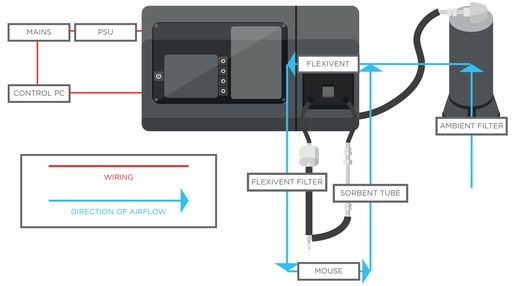

Figure 1 - *A schematic showing the mouse breath sampling system used for this study. This system consists of a flexiVent small animal ventilator, an ambient filter, and a flexiVent filter both connected to the ventilator by flexible tubing, and a sorbent tube for breath collection.*

3. Results

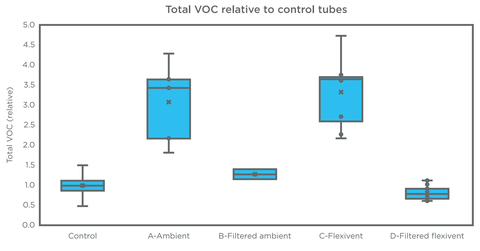

Figure 2 - *The different levels of total relative VOC concentration in different blank samples to test the background of the flexiVent system. The control was an empty tube, A was ambient air in the room, B was the filtered ambient air, C was the air in the flexiVent system, and D was the filtered air in the flexiVent system.*

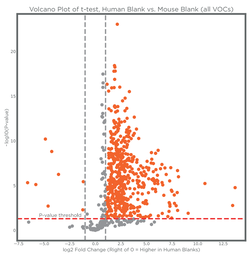

Figure 3 - *Volcano plot comparing all VOCs in human and mouse blank samples.*

To optimize untargeted analysis, a clean background is necessary to maximize the signal-to-noise ratio. Results (Figure 2) show that a second filter removes flexiVent contamination, identifying signals of interest more easily.

When comparing background signal between human blanks and mice blanks, 498 out of 661 (75.34%) features were significantly different. Most of the human blanks have higher signal than mice blanks, demonstrating flexiVent system have sufficiently lower background contamination levels for untargeted analysis of mouse breath (Figure 3).

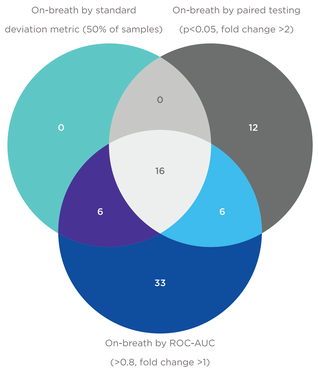

Figure 4 - *The number of overlapping identified "on-breath" VOCs by each metric type.*

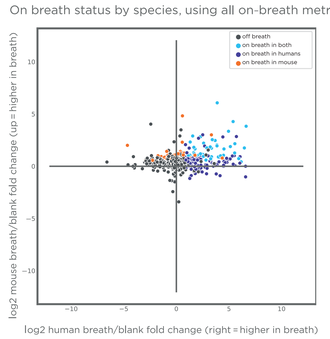

Figure 5 - *A plot of the on-breath compounds identified in mouse breath and human breath by fold change compared to blank concentration. The off-breath compounds are shown in grey, those only in mice in orange, those only in humans in purple, and the on-breath compounds in both mice and humans are shown in blue.*

Depending on which classification thresholds were used, 16 – 73 on-breath VOCs were identified in mice (Figure 4). To assess translatability, we compared and found 57 common on-breath VOCs between mouse and human (Figure 5). While more VOCs were classified as on-breath in humans, this was expected due to the differences in relative size, lung volume, and environmental exposure between mice and humans.

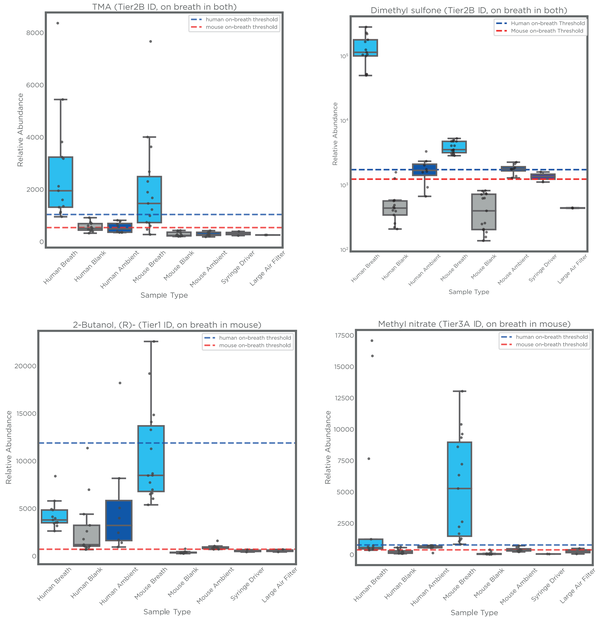

Figure 6 - *Examples of identified compounds. Trimethylamine (TMA) and dimethyl sulfone are identified on-breath in both humans and mice. 2-Butanol and methyl nitrate are on-breath in mice only.*

Examples of compounds common to mouse and human breath include TMA and dimethyl sulfone. Some compounds appear to be exclusively on-breath in mice only, including methyl nitrate and 2-butanol, both with a high signal intensity. These VOCs rarely observed in humans may represent a species-specific difference in breath composition or reflect a difference in environmental or dietary exposure. The VOC profiles demonstrate the compounds seen in healthy populations. Future studies should involve the breath analysis and profiling of patients and mice with ILD/IPF.

4. Conclusions

- The results of this study present a reliable mouse breath sampling and analysis platform that can be used to compare the composition of mouse breath with human breath and establishes mice as a viable animal model for the pre-clinical study of breath biomarkers, particularly in the context of respiratory disease.
- We identified 472 compounds in mouse breath, and 73 (15.47%) of these were considered as 'on-breath'. This demonstrates our three quantitative metrics are capable to distinguish signals that suggest "on-breath" compounds from background contamination.
- When comparing on-breath compounds, 57 (29.08%) were common between mouse and human, and some were linked to suspected biological functions.
- The data found in this study can be used as a benchmark to compare diseased subjects to, in order to identify disease biomarkers of interest.

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