Development of an *in vitro* Model for the Identification of Volatile Biomarkers Differentiating *Streptococcus pneumoniae* Infection of Human Lung Cells

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Introduction

Pneumonia remains one of the leading causes of global mortality affecting 2.49 million people annually due to poor diagnostic measures [1]. In recent years, volatile organic compounds (VOCs) have emerged as easily accessible biomarkers for use in point-of-care diagnostics. Despite recent advancements, exhaled breath analysis studies have demonstrated poor predictive accuracy with VOCs falsely discovered due to noise or contamination [2]. Through this work, we aim to utilize custom technology to collect VOCs released exclusively by pneumonia-causing bacterial strains in the presence of small cell lung cancer cells with dramatically reduced noise levels.

Experimental Design

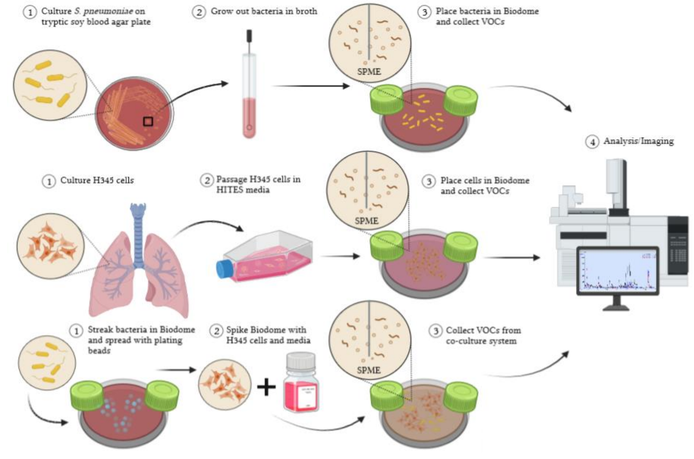


Figure 1. Schematic of experimental process of pneumonia, H345 cell, and co-culture conditions.

Experimental Set-Up and Image Analysis

Figure 2. Experimental set-up of Biodome with solid tryptic soy agar layer on the bottom and H345 cells in liquid medium on the top. Biodome is attached to a SPME fiber and placed in a carbon bead bath for data collection.

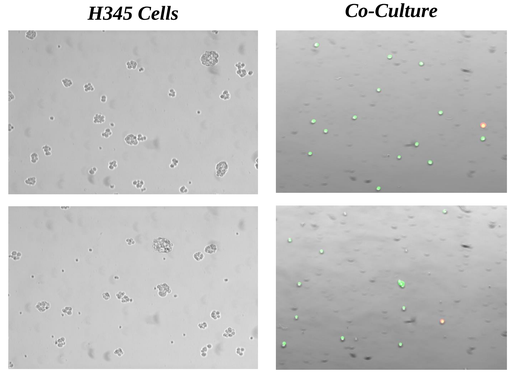
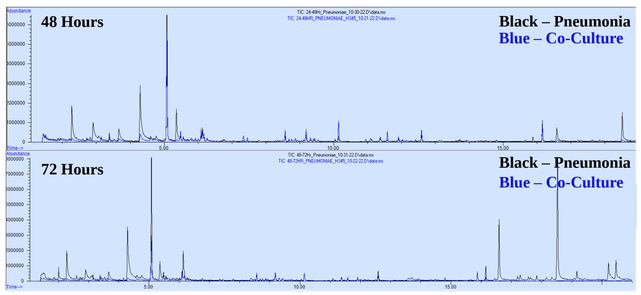


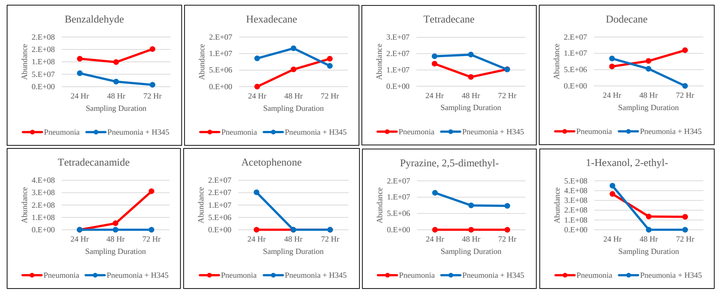
Figure 3. Bright field images of H345 cells after 48 hours (left) at 10X objective lens and LIVE/DEAD assay images of H345 cells in the co-culture system after 48 hours (right).

Results

(a) Mass Spectrometry Chromatograms



(b)



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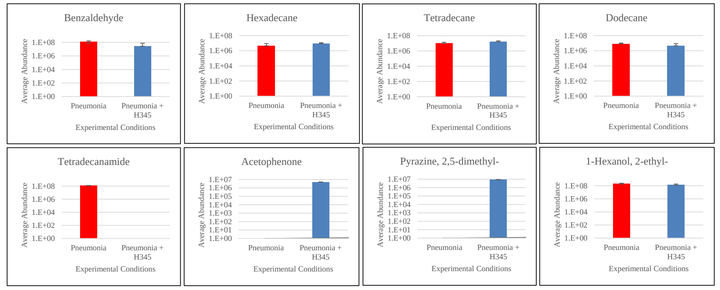

Figure 4. (a) Chromatogram overlays of Pneumonia only and Pneumonia+H345 (co-culture) conditions after 48 hours (top) and 72 hours (bottom). (b) Line plots of the integrated abundances in VOCs found in Pneumonia only and co-culture conditions across 72 hours. (c) Bar graphs of the average abundance in VOCs found in Pneumonia only and co-culture conditions from all three time points. The eight VOCs include Benzaldehyde, Hexadecane, Tetradecane, Dodecane, Tetradecanamide, Acetophenone, Pyrazine,2,5-dimethyl-, and 1-Hexanol,2-ethyl-. The data was processed through R Studio Program and the line and bar plots were developed through Excel.

Discussion/Conclusion

In this study, a successful physiologically relevant model was developed to collect VOCs from *S. Pneumoniae* bacteria in the presence of human lung cells. There were several VOCs that were found including Benzaldehyde, Hexadecane, Tetradecane, Dodecane, Tetradecanamide, Acetophenone, Pyrazine,2,5-dimethyl-, and 1-Hexanol,2-ethyl-. In particular, Pyrazine,2,5-dimethyl- and Acetophenone was evident only in the co-culture condition, possibly indicating potential biomarkers specific to Pneumonia.

Acknowledgements

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References

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2. van Oort, P. M. P., de Bruin, S., Weda, H., Knobel, H.H., Schultz, M. J., & Bos, L. D. (2017, February 19). Exhaled breath metabolomics for the diagnosis of pneumonia in intubated and mechanically-ventilated intensive care unit (ICU) Patients. International journal of molecular sciences. Retrieved October 9, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343983/

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