Development of an *in vitro* Model for the Identification of Volatile Biomarkers of Pneumonia
Paula Phan, Biomedical Engineering
Mentor: Barbara Smith, Ph.D.
School of Biological Health Systems Engineering

**Research Question**
The objective of this research is to identify biomarkers of Pneumonia by developing a co-culture system using custom technology and examining its volatile organic compound expression across time.

**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**

![Diagram of experimental process of pneumonia, H345 cell, and co-culture conditions.](image)

**Results**

![Experimental set-up and image analysis.](image)

**Mass Spectrometry Chromatograms**

![Chromatogram overlays of significant VOCs found in pneumonia only, H345 only, and co-culture conditions after 48 hours (top) and 72 hours (bottom).](image)

**VOC Modeling Analysis**

![Bar graphs of the average abundance in significant VOCs found in pneumonia only, H345 only, and co-culture conditions.](image)

**Figure 2.** (a) Experimental set-up of inoculation with SPME fiber and carbon bead bath for data collection. (b) Bright field images of H345 cells after 48 hours (left) and LIVE/DEAD assay images of H345 cells in the co-culture system after 48 hours (right).

**Figure 3.** Chromatogram overlays of significant VOCs found in pneumonia only, H345 only, and co-culture conditions after 48 hours (top) and 72 hours (bottom).

**Figure 4.** (a) Bar graphs of the average abundance in significant VOCs found in each condition across 72 hours.

![Mass spectrometry chromatograms showing integrated abundance of significant VOCs found in each condition across 72 hours.](image)

**Conclusion**
In this study, we successfully developed a physiologically relevant model to collect VOCs from pneumonia-causing bacteria in the presence of small cell lung cancer cells. Benzaldehyde was a VOC that was found in the pneumonia and co-culture samples, but not in the normoxic H345 sample. This may indicate a potential VOC associated with pneumonia that is worth investigating further.

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**References**

[1] https://ourworldindata.org/pneumonia