

**Official Title:** Acute Effects of Low Temperature Exposure on Respiratory System

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## Statistical Analytical Plan

## **1. Health outcomes related to respiratory system**

### **1.1 Linear mixed-effects model**

A linear mixed-effects (LME) model would be utilized explore the effects of low temperature on lung function. We assessed the differences of changes in post-exposure measures between the intervention and control groups using LME model. To account for potential confounding effects, several confounders would be included in the LME model, including gender (male, female), age (years), and body mass index (BMI, kg/m<sup>2</sup>). Additionally, random intercepts for each participant would be included to account for the repeated measurements. Similarly, LME model would be used to examine the effects of low temperature on airway biomarkers, the biomarkers in blood samples and the biomarker in exhaled breath condensate, based on post-exposure metrics comparing the intervention and control groups.

### **1.2 Analytical tools**

The 16S rRNA sequences of respiratory microbiota from pharyngeal secretion samples were analyzed using the Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline for microbial community analysis. All statistical analyses are carried out in R software version 4.1.1. Statistical tests are two-sided, and  $p < 0.05$  is considered statistically significant.

## **2. Omics analysis**

All the omics analyses are performed in strict accordance with the manufacturer's guidelines. All omic data were log-transformed before formal analyses. We mainly apply the linear mixed-effects model to analyze the percent changes of detected omics features associated with low temperature exposure. The Benjamini-Hochberg procedure was employed to estimate the false discovery rate (FDR) using  $p$  values obtained from the LME models. Differential analytes are further conducted based on the  $p$  values obtained from the LME models. For the identified differential features from the LME model, pathway enrichment is performed at the ingenuity pathway analysis platform (IPA, QIANGEN, Germany). The pathways with FDR of less than 0.05 are considered statistically significant.