# Developing a Breath Test for Valley Fever using GC×GC Untargeted Metabolomics

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Valley fever (coccidioidomycosis) is an endemic pneumonia of the North and South American deserts, and is responsible for up to 30% of community-acquired pneumonias in endemic and highly populated areas of the US desert southwest. The causative agents of Valley fever are the dimorphic fungi *Coccidioides immitis* and *C. posadasii*, which grow as mycelia in the environment and spherules within the lungs of vulnerable hosts. The current diagnostics for Valley fever are severely lacking due to poor sensitivity and invasiveness, contributing to a 23-day median time-to-diagnosis. Our long-term goal is to develop a sensitive, non-invasive diagnostic for coccidioidomycosis through a breath test.

Thus far we have characterized the volatile organic compounds (VOCs) produced by *Coccidioides immitis* and *C. posadasii in vitro* and evaluated the relationship of the volatile metabolomes to lifecycle, and we have investigated the VOC profiles of bronchoalveolar lavage fluid (BALF) samples from mouse model lung infections of Valley fever. For *in vitro* analyses, six strains each of *C. immitis* and *C. posadasii* were cultured in triplicate to induce mycelial or spherule formation. For mouse model infections, three cohorts of mice were infected by intranasal inoculation with *C. immitis* RS (n = 6), *C. posadasii* Silveira (n = 6), or vehicle control (n = 4), and BALF fluid was collected 10 days post-infection. The *in vitro* spent media and BALF sample VOCs were analyzed by headspace solid-phase microextraction and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (SPME-GC×GC-TOFMS). The volatile metabolomes were compared using a variety of statistical analyses.

We detected a total of 353 VOCs that were at least two-fold more abundant in a *Coccidioides* cultures versus medium controls and found the volatile metabolome of *Coccidioides* is more dependent on lifecycle than species. The BALF samples indicate that lung infection VOCs are correlated to cytokine production and classify mice based on their individual level of infection. Combined, these studies suggest that *Coccidioides* spp. and the host produce volatile metabolites that may yield biomarkers for a Valley fever breath test. The next steps of this work will be to collect the volatile metabolites from lung specimens (BALF and sputum) from persons with community-acquired pneumonia, and determine which of the *in vitro* and murine model Valley fever biomarkers can differentiate between bacterial and fungal etiologies of disease.