

## Detection and Differentiation of *Mycobacterium avium* complex Species Using Proteomic Based Discovery: LSU Pilot Proof of Principle Phase 1 Study

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**Rationale:** Over the last few years, the awareness and the need for treatment and management of nontuberculous mycobacteria (NTM) pulmonary disease has been increasing. The worldwide distribution of NTM respiratory disease has now been well documented with *Mycobacterium avium* pulmonary infection being the predominant pathogen.<sup>1,2</sup> The prevalence of this infection in the coastal belt of US has been reported between 150–200 per 100,000 people, this being 70% of all NTM cases in US. In the US it is projected that we will be seeing an 8% annual increase in its prevalence.<sup>3</sup> Other than the previously recognized risk factor in cystic fibrosis, HIV and dual infection seen with tuberculosis, we are now recognizing this infection in COPD, pulmonary fibrosis, sarcoidosis, and associated with gastroesophageal reflux disease, naso-sinusitis, rheumatological disease states, primary bronchiectasis and Cystic Fibrosis Carriers. Smoking and environmental exposure remain as associated risk factors. Criteria of diagnosis of NTM pulmonary infection notwithstanding, NTM infection is a product of the pathogenicity of the mycobacteria, load and duration of infection, and the host response based on underlying disease, co-morbid states, degree of airway and systemic immunosuppression of the host and other genetic factors.

Nontuberculous mycobacteria (NTM), specifically *Mycobacterium avium* complex (MAC), has become an important cause of pulmonary disease worldwide.

Diagnostic methods and clinical correlation with routine cultures, however, remain imprecise with delayed turnaround time and MAC generally reported as a group non-specific diagnosis. While advanced diagnostic molecular methods are available, they are not readily implemented due to high cost and delayed results.

## **Background**

Proteomic analysis is a novel yet proven diagnostic modality utilizing advanced mass spectrometry techniques to identify proteins specific to an organism. Proteins from two strains were isolated for the profiling of the entire proteome. The steps include:

***Trypsinization*** – Incubation with Trypsin endopeptidase cleaves the unknown proteins at the C-terminal side of Lysine (K) and Arginine (R) residues producing an array of peptides specific to the protein of interest.

***Liquid Chromatography Mass Spectrometry (LCMS)*** – This mixture is then subjected to liquid chromatography which helps simplify the mixture before introduction into a mass spectrometer. The experimentally obtained spectra is compared to an in silico “digested” database for possible matches. While each strain produces thousands of spectra, an example spectrum is shown (Figure 1)

## **Methodology**

▪ ***Discovery Phase*** – As a proof-of-principle, we initially sought to profile two strains for comparison to determine if we could differentiate between the strains via LCMS. We chose *Mycobacterium avium* (ATCC) and *Mycobacterium intracellulare* (ATCC) as reference strains. Both strains were isolated from sputum from infected human patients. These strains were culture in Middlebrook 7H9 broth with ADC enrichment and glycerol for 21 days and the protein fraction was extracted. The protein fractions were subjected to the LCMS techniques described. In the Discovery-Phase, the raw LCMS data was compared to all known protein sequences for NTM sub species; a total of over 30,000 proteins. From all possible matches, only unique sequences were attributed to either strain. We were able to detect a total of 385 high scoring proteins unique and specific to *Mycobacterium avium* and 747 high scoring proteins unique and specific to

*Mycobacterium intracellulare*. Some of the most abundant proteins unique to either strain included ubiquitous proteins such as 30S ribosomal proteins, ATPases and other metabolic enzymes, and nucleic acid synthesis and replication proteins. The next step is to culture and extract proteins from the remaining strains, *M. chimaera* and *M. colombiense* of the MAC spectrum and identify those unique and specific proteins. These sets will then be combined to produce a list of unique proteins that will be tested in the Targeted-Phase.

- **Targeted Phase** – When the profiling has been completed, we will curate our newly created biospecimen databases to identify protein ‘targets’ that are both unique and specific to the individual strains. We will develop a Parallel Reaction Monitoring (PRM) protocol that can identify these targets in complex mixtures using LCMS. This PRM method will be thoroughly tested in the research lab to ensure its effectiveness. During PRM, only selected unique and specific peptides will be monitored, increasing specificity and sensitivity. In addition to detection, quantitation can be performed as well. Peptides are “filtered” at the MS1 stage, and those targets are fragmented into transitions specific to the peptide sequence. Those transitions are measured in the Orbitrap. Subsequent analysis reveals both identity and quantity of the relevant peptides. ( Figure 2 )
- **Implementation Phase** – We will then use the PRM technique to analyze and diagnose patient samples. These diagnoses will then be verified through further culturing and taxonomy identification.
- **Clinical phase** –Following exhaustive testing, we hope to scale the technique for daily and routine testing in laboratories

## Conclusion

Protein Profiling has been performed on *M. avium* and *M. intracellulare* determining that the two strains can be differentiated using LCMS, with over 1000 unique high scoring peptides. We will continue to develop the remaining profiles of *M. chimaera* and *M. colombiense*. In the Targeted-Phase, we will develop a

strategy using PRM to selectively monitor unique peptides to each strain for both qualitative and quantitative information.

**Baseline Reference also enclosed ; More references available on request**

**Future Directions:**

Using these protein and peptide identification techniques, we can potentially develop an ELISA based clinical diagnostic tool which can be complementary to current diagnostic tools, albeit with a rapid turnaround time. This process can also be extended for the differentiation of *Mycobacterium Abscessus*.

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