

January 2, 2013

## Implementation of *rpoB* and 16S rRNA gene sequencing for identification of NTM; discontinuation of HPLC

Dear Client:

Effective February 1, 2013, National Jewish Health® Advanced Diagnostic Laboratories will no longer utilize the current high-performance liquid chromatography (HPLC) method of testing for the identification of mycobacteria. Instead, we will utilize two molecular assays: *rpoB* gene and 16S rRNA gene sequencing.

Nontuberculous mycobacterial infections are increasing in frequency in many parts of the world, including the United States. Currently, there are more than 150 different species of nontuberculous mycobacteria (NTM) described that differ in their virulence and in response to antibiotic treatment. Due to these differences, accurate identification of isolated NTM is important.

For decades, NTM were identified by phenotypic characteristics (pigmentation, growth rate) combined with biochemical tests or analyses of cell wall mycolic acid content by HPLC. These have now been largely replaced by molecular methods, including DNA probes and direct sequencing, which have reduced the turnaround time for identification from weeks or months to days.

Sequencing offers the highest level of resolution, provided that suitable target genes are used. Initially, the 16S rRNA gene was considered the most suitable target. In recent years, distinct species have been described that share identical 16S rRNA sequences, resulting in a search for more useful targets. Of these, the gene encoding the beta subunit of the bacterial RNA polymerase (*rpoB*) has provided a reasonable level of discrimination among species and is increasingly used in microbiology laboratories. This target has proven useful mainly in the *M. avium* complex and the rapidly growing NTM, including the *M. abscessus* and *M. fortuitum* complexes; combined, these make up about 90% of all NTM isolates identified by National Jewish Health.

If *Mycobacterium tuberculosis* complex has not yet been ruled out by the submitting laboratory, our laboratory will do so by AccuProbe and follow up with final identification and drug susceptibility testing. Once *M. tuberculosis* complex has been ruled out, *rpoB* gene sequencing is used for NTM and 16S rRNA gene sequencing for other genera that resemble NTM such as *Nocardia* and *Rhodococcus*.

The cost is \$290 each for the *rpoB* gene and 16S rRNA gene sequencing, and these tests will be run two times a week.

We are updating our mycobacteriology requisition form to reflect the test changes referenced in this letter. You will receive the new form before February 1, 2013.

We appreciate your continued support of National Jewish Health Advanced Diagnostic Laboratories. Please contact our mycobacteriology team at 303.398.1339 or TBLab@njhealth.org if you have any questions or would like additional information regarding these changes.

Sincerely,



Max Salfinger, MD, FAAM, FIDSA  
Laboratory Director, Mycobacteriology