16S rRNA Gene Sequencing for Identification of Bacteria from Culture Isolates

Summary and Explanation of the Test:

Identification of certain groups of bacteria utilizing traditional methods (growth characteristics and biochemical reactions) can be difficult for certain groups such as non-fermenters, mycobacteria, nocardia and other rarely isolated organisms. DNA sequencing of ribosomal RNA genes has become an attractive alternative to traditional methods for identifying these bacteria. The ribosomal RNA genes exhibit markedly variable sequences when comparing between different species. These genes also have highly conserved sequences between all bacteria, and these regions are used as primer binding sites for amplification and sequencing reactions. Bacteria genomic DNA is extracted from a pure culture and the 16S rRNA gene amplified using PCR primers that target a ~1500 base pair region. Sequencing is performed in the forward direction with the same primer as used in the amplification reaction. Sequencing results are used to search databases (Microseq, Genbank, and our own UTMC database) of verified bacterial sequences and the percent match to the top hits is calculated and evaluated along with phylogenetic trees of relatedness. These results are compared to the traditional methods used in identification of the organisms as well as clinical and publication-related data.

Turn-Around-Time: 5 days

Sample Requirements:

1) PURE bacterial culture isolate on an agar slant, culture plate or broth; OR
2) Patient sample submitted to UTMC for culture first, and then if positive, the pure isolate will be sequenced.

Results Reporting:

An official report is issued describing the results with an interpretation.

References:

1) CLSI. *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline.*CLSI document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. 2) She RC, et al. Chapter 30, Identification of Bacteria by DNA Target Sequencing in a Clinical Microbiology Laboratory, p479-489. In D. H. Persing, F. C. Tenover, Tang Y-W, Nolte FS, Hayden RT, Van Belkum A. (2nd, ed.), Molecular Microbiology: Diagnostic Principles and Practice, 2011, ASM Press, Washington D.C. 3) Hall L, Doerr KA, Wohlfiel SL, Roberts GD. Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. *J Clin Microbiol.* 2003;41:1447-1453.

For any questions regarding bacterial sequencing identification or other molecular diagnostics testing, please contact the Molecular Diagnostics laboratory at 419-383-5636 or the medical director at 419-383-6444. Further information can also be found on the Molecular Diagnostics web site at: http://www.utoledo.edu/med/depts/path/moldx/index.html