

## Fungal rRNA Gene Sequencing For Identification

### Summary and Explanation of the Test:

Identification of certain fungi utilizing traditional methods (growth characteristics, morphology and biochemical reactions) can be difficult. DNA sequencing of ribosomal RNA genes has become an attractive alternative to traditional methods for identifying these fungi. The ribosomal RNA genes exhibit markedly variable sequences when comparing between different species. These genes also have highly conserved sequences between all fungi, and these regions are used as primer binding sites for amplification and sequencing reactions. Fungal genomic DNA is extracted from a pure culture and the ITS1, ITS2, 5.8S, and 28S D1/D2 rRNA genes are amplified using PCR primers that target a ~1450 base pair region. Sequencing is performed in the forward direction with primers that are either the same as used in the original PCR reaction or that are nested within the region of amplified DNA. Sequencing results are used to search databases (Microseq, Genbank, and our own UTMC database) of verified fungal 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:** 7 days

### Sample Requirements:

- 1) PURE fungal culture isolate on an agar slant or culture plate, 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. (2<sup>nd</sup>, 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 fungal sequencing identification, please contact the Molecular Diagnostics laboratory at 419-383-5636 or the director at 419-383-6444. Further information can also be found on the Molecular Diagnostics web site (including a requisition):

<http://www.utoledo.edu/med/depts/path/moldx/index.html>.