Identification of Nonsporulating Molds by Sequencing Internal Transcribed Spacer Regions with Virodec Software and Database

J. I. Pounder[1], K.E. Simmon[1], C. A. Barton[1], S. L. Hohmann[1], C.A. Petti[1,2]
[1]ARUP Laboratories, Salt Lake City, UT; [2]University of Utah School of Medicine, Salt Lake City, UT

Background: Fungal infections are increasing, particularly among immunocompromised hosts, and a rapid, accurate diagnosis is essential for the initiation of targeted antifungal therapy. Identification of fungi from culture requires the presence of reproductive structures, and the absence of spores can increase the time to identification up to 21 days. We evaluated the utility of amplification and direct sequencing of internal transcribed regions ITS I and ITS II from cultures with nonsporulating molds. **Methods**: Fifty nonsporulating molds from clinical isolates were randomly selected. After growth on potato dextrose agar, DNA was extracted from approximately 1 cm^2 mycelia with the IDI lysis kit. Using ITS1 and ITS4 primers, real-time PCR with SYBR green DNA binding dye followed by melting temperature analysis was performed on RotorGene 3000. Amplicons were sequenced using BigDye chemistry on ABI 3130. Sequences were identified using Roche Virodec™ software. Nucleotide sequences with match length of $\geq$ 400 bp were analyzed. Sequence-based identifications were defined by percent similarity: species $\geq$ 99%, genus $\geq$ 93%, and inconclusive $\leq$ 92%. **Results**: Forty-eight of 50 isolates had $\geq$ 400 bp match length (mean 574 bp). Of this group, Virodec identified 9 (19%) to genus only, 35 (73%) to species with 4 (8%) being inconclusive. Seventeen of 48 sequences shared similar nucleotide sequences with multiple species. Classification of molds was as follows: 25 plant or soil-associated, 8 well-recognized pathogens, and 11 potential/emerging pathogens. Wide variability within ITS regions was observed with several reference sequences for the same microorganism. **Conclusions**: Sequencing the ITS regions identified 88% of nonsporulating molds that could not be identified by conventional methods. Moreover, a significant number of these molds were well-recognized or emerging pathogens for immunocompetent and immunocompromised hosts, and their identification potentially may have impacted patient management. Virodec software was useful for sequence analysis, and a more thorough evaluation of this database is currently ongoing.

Research: Pre-Submission Approval Form

Approval is required **before information is presented outside of ARUP and enters the public domain** to ensure that HIPAA and IRB protocols have been followed. Please ensure that this document is signed and appropriate documents are attached before submitting any information for publication/presentation outside of ARUP.

☐ Attach copy of (please indicate) manuscript, poster, abstract, or other presentation

Presentation/Poster presented at (specify meeting or conference): _____

ARUP Cited: Yes ☐ No ☐ If no, state reason: _____

☐ Global IRB #7275 applies, and PRCS-0020, *Internal Sample Request: De-Identification of Samples* has been followed **OR**

☐ Independent Institutional Review Board (IRB) approval, IRB# _____
Attach copy of approved IRB protocol.

OR

☐ IRB is not applicable. Please explain: _____

Scientist/Researcher: _____ Date: _____
Signature

Approval Signatures and Dates:

Medical Director: _____ Date: _____

R&D Group Manager or
ARUP Privacy Officer: _____ Date: _____

CONFIDENTIAL: This material is prepared pursuant to Utah Code Annotated, 26-25-1, et seq., for the purpose of evaluating health care rendered by hospitals or physicians and it NOT PART of the medical record.