

## PCR / SEQUENCING FOR IDENTIFICATION OF PATHOGENIC FUNGI

\*Boyington C, Deneer, H., Mochoruk K, Traiforos E, Sanche SE  
Department of Medicine, University of Saskatchewan, Saskatoon,  
Saskatchewan, Canada

Invasive fungal infections are recognized to be important causes of morbidity and mortality in immunocompromised patients. Given the limitations of culture and histology, molecular methods are being used with increasing frequency to improve sensitivity, accuracy and turnaround time of mycologic diagnosis. We previously described a PCR method for differentiation of pathogenic fungi based on amplification of the Internal Transcribed Spacer 2 (ITS2) region with subsequent determination of product length using capillary electrophoresis. We decided to try sequencing the PCR product because a number of organisms had similar PCR product length and thus could not be accurately identified using the original method.

**Methods:** DNA was isolated from pure cultures of organisms (previously identified by conventional methods) by cell disruption using glass beads in conjunction with a commercial DNA isolation kit. After the PCR products were quantified and sequenced, GenBank Blast searches were performed, and the sequence- based and conventional identifications were compared.

**Results:** To date, excellent yeast identifications have been obtained for multiple *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* ) and *C. albidus*. Moulds tested to date include *A. fumigatus*, *A. nidulans*, *P. boydii*, *F. oxysporum*, and *F. napiforme*.

**Conclusions:** PCR / sequencing of the ITS2 region can be used to identify pathogenic fungal organisms in pure culture. With modification, this method might also prove useful for detection and identification of fungi from human tissues.