

O217

Oral Session

Advances in molecular mycology: notes from the bench

**DEVELOPMENT AND EVALUATION OF A NOVEL MULTIPLEX ASSAY USING RENDX® SURFACE ENHANCED RESONANCE RAMAN SCATTERING (SERRS) TECHNOLOGY TO AID IN THE DIAGNOSIS OF INVASIVE FUNGAL DISEASE**

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- Objectives

Early diagnosis of invasive fungal disease (IFD) remains difficult, and empirical therapy is used to reduce mortality. PCR based diagnosis has been shown to be a sensitive early indicator of infection, but suffers through a lack of standardisation. Commercially developed, quality controlled assays would alleviate this, but are limited to specific pathogens or have minimal clinical evaluation.

SERRS is a powerful analytical tool capable of sensitive detection of analyte-specific fingerprint spectra, suited to the simultaneous detection of multiple targets beyond the capabilities of real-time PCR. RenDx® technology was used to develop a commercially controlled, semi-automated multiplex PCR-SERRS assay detecting *Candida/Aspergillus*, while differentiating potentially resistant species. The objective of the study was to determine the analytical and clinical performance of this assay, comparing it with already established real-time PCR diagnosis.

- Methods

The Fungiplex assay was designed to detect *Aspergillus/Candida* species, but also differentiate *A. terreus*, *C. krusei* and *C. glabrata*. The analytical and clinical performance of the assay was determined across two centres. One centre performed DNA extraction/PCR amplification, before the second centre performed the Fungiplex assay blinded to sample identity. Analytical validity was determined by testing replicates of quantified plasmid concentrations ( $10^6$  to  $<10$  input copies) and 100 simulated samples (EDTA whole blood [n=25], serum/plasma [75]) containing 100-10 genomes per sample. Clinical validity was determined by testing DNA extracts (n=111) from samples submitted for routine fungal PCR investigations, with disease defined according to the EORTC/MSG criteria, and independent of the PCR result. The result was compared to the real-time PCR result and clinical performance parameters determined.

- Results

The limit of detection (LOD) for PCR/SERRS was 20 plasmid copies for all targets, corresponding to less than 1 genome of each target. Only five of 108 negative amplification controls generated false positive results and were attributed to environmental / handling errors.

For simulated samples the LOD was 10 genomes per sample. None of the eight un-spiked samples generated a false positive result. Performance was similar across the pathogen range.

In testing 51 clinical samples from 34 patients diagnosed with IFD, the following sensitivities were determined:

Invasive candidosis: 73.3%, (48.1-89.1)

Proven/probable aspergillosis: 78.9%, (56.7-91.5)

Combined sensitivity: 76.5%, (60.0-87.6)

Only 3/60 samples from patients without evidence of IFD were positive. generating a specificity of 95% (86.3-98.3)

Results were comparable with the real-time PCR assays, with the advantage of a broader target range.

- Conclusion

The RenDx Fungiplex assay is robust, semi-automated, commercially produced and validated, providing multiplex performance comparable to monoplex real-time PCR systems.

The multiplexing capabilities allow detection of numerous pathogens improving patient management, through tailored therapy where required.

The excellent clinical performance is likely to be enhanced by prospective evaluation.

The high specificity provides confidence in diagnosing IFD.