Evaluation of PCR electro-spray ionization-mass spectrometry (PCR/ESI-MS) Fungal Spectrum Assay

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Introduction

The accurate identification of invasive fungal pathogens remains a significant challenge for the clinical laboratory. The culture and subsequent identification of these pathogens can take several weeks to perform[1].Techniques such as PCR that target conserved fungal sequences, such as the Internal Transcribed Spacer region (ITS)[2], have proved to be a useful aid to diagnosis.

The Fungal Spectrum (FS) assay (Ibis Biosciences, Abbott, Carlsbad, CA, USA) incorporates simultaneous broad-range multilocus PCR amplification of conserved DNA regions, coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) to identify fungal DNA. Particular loci are targeted to better discriminate fungal species more commonly associated with clinical significance (i.e. genera *Candida and Aspergillus*)[3].

PCR/ESI-MS was evaluated as a novel means for the identification of fungal pathogens.

Materials and Methods

•199 previously identified fungal cultures were obtained from the Mycology Reference Centre, Leeds General Infirmary for analysis using PCR/ESI-MS.

•A wide range of genera and species comprising of 70 yeast and 129 molds were selected with particular emphasis on clinically relevant species.

•All isolates were extracted and processed using Abbott equipment, reagents and protocols as recommended for use with PCR/ESI-MS assays.

•DNA from specimens was processed using the FS assay and analysed using the Non-Diagnostic reporting software for research use only (RUO).

•Discrepancies with fungal identification were resolved using repeat phenotypic methods and sequence data from the 18S-ITS-25S region.

Table 1. Tested fungal cultures

Organism	No
Aspergillus spp	29
Fusarium spp	47
Other molds	53
Candida spp	54
Cryptococcus spp	6
Exophiala spp	4
Other yeasts	6

Results

• The FS assay assigned 87% of molds and 97% of yeasts to the correct genus.

• 61% of molds and 86% of yeasts were correctly identified to species level.

• All isolates from the genera *Aspergillus*, *Fusarium* and *Candida* were identified correctly to genus level and 83%, 74% and 91%, respectively, were identified to the correct species.

• Multiple species were reported for 18 (9%) isolates with the PCR/ESI-MS and a single incorrect genus was reported on 13 (6.5%) occasions.

• The PCR/ESI-MS did not record the detection of 4 molds even upon repeat tesing (*Acremonium strictum x2, Trichophyton terrestre* and *Ochroconis galloparva*).

Table 2. Comparison of PCR-ESI/MS results with reference organisms .

(n)	Correct genus	Correct species
Molds (129)	112	79
Yeasts (70)	68	61
Aspergillus spp *(29)*	29	24
Fusarium spp (47)	47	35
Candida spp (54)	54	49
Cryptococcus spp (6)	6	5

Conclusions

• The FS assay performed very well identifying yeast species. The isolates misidentified from the genus *Candida* were uncommon species (*C. auris*, C.*metapsilosis* and C.*haemulonis*.)

• The majority of commonly isolated molds were correctly identified.

• The majority of fungal isolates misidentified to genus were obscure fungi not included in the RUO database.

References & Acknowledgement

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