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P2201 The development of a real-time PCR assay for the detection of Mucormycosis infections: Fungiplex mucorales

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Background: Mucorales have been increasingly reported as causes of invasive fungal infections in immunocompromised subjects, particularly in patients with haematological malignancies, uncontrolled diabetes mellitus or those undergoing dialysis. Mucorales are now also being reported in *Aspergillus*-positive patients who are not responding to first line treatments.

Histology and culture are still the most important diagnostic approaches for mucormycosis because of the lack of molecular diagnostic methods available, and β-d-Glucan detection is not useful due to the extremely low content of the biomarker in the Mucorales order. Timely diagnosis of invasive mucormycosis is essential due to the rapid progression of the disease, and because signs and symptoms of the infection are consistent with other invasive fungal infections. Mucorales lacks diagnostics and it is suspected that prevalence is higher than reported, therefore the use of PCR could improve the sensitivity of Mucorales detection and influence appropriate treatment decisions more rapidly.

Materials/methods: Universal primer and probe sequences have been designed to target the internal transcribed spacer (ITS) region of the rRNA gene for the genera detailed in Table 1. An internal control and extraction control has been developed for the assay to monitor sample inhibition. The Bruker real-time PCR assays are designed in an easy to use format with minimum hands on time and results generated in less than 2 hours from extraction.

Table 1: Genera detected by the Fungiplex Mucorales Real-Time PCR Kit

Lichtheimia spp.	***Rhizopus spp.***	***Mucor spp.***
Cunninghamella spp.	*Rhizomucor spp.*	*Apophysomyces spp.*
Saksenaea spp.	*Syncephalastrum spp.*	*Actinomucor spp.*

Results: The coverage specified in Table 1 has been confirmed using a range of simulated samples prepared from plasmid and genomic DNA. The limit of detection for species representing the highest prevalence of IFD for each genus has been assessed over six thermocycler platforms to ensure accuracy across a variety of systems.

Conclusions: Bruker have developed a Mucorales real-time PCR assay which identifies a wide range of clinically relevant genera. The assay is being further developed to produce a kit to provide rapid detection of the main causative agents of invasive mucormycosis.