28th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Madrid, Spain
21 – 24 April 2018

P0178 The development of a real-time PCR assay for the detection of azole resistance in Aspergillus fumigatus-positive patients

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Background: Bruker aim to expand their portfolio of real-time PCR assays within the area of invasive fungal disease (IFD), allowing a more tailored diagnostic approach, and supporting the needs of a specific patient population through a single product range.

Azoles are the primary therapy in the treatment of Invasive Aspergillosis (IA). As the incidence of IA increases, an increasing amount of secondary azole resistance is reported in *Aspergillus fumigatus* strains. *A. fumigatus* is difficult to culture from positive clinical samples, therefore, a PCR assay for the detection of azole resistance would improve diagnosis and guide patient therapy. Bruker are developing an *Aspergillus* azole resistance real-time PCR assay targeting the TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in the *cyp51A* gene as a companion product to *Fungiplex Aspergillus*.

Materials/methods: 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. Universal primers and specific probes have been designed to target the tandem repeat associated with the TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in the *cyp51A* gene.

Results: A range of known plasmid concentrations were tested with the Aspergillus azole resistance real-time PCR assay resulting in a reproducible limit of detection of 20 input copies for both the TR_{34} and TR_{46} targets. Table 1 shows the resultant Ct values for each target and corresponding internal amplification control (IAC) when tested on the QuantStudio 5 thermal cycler.

Table 1: Results for TR_{34} and TR_{46} plasmid targets at known concentrations

Plasmid concentration (ipc)	Average Ct			
	TR_{34}		TR_{46}	
	Target	IAC	Target	IAC
20	33.3	33.9	37.2	33.4
50	32.5	33.6	34.9	33.3
200	30.1	34.7	33.3	33.2
$2X10^3$	26.7	38.6	29.9	33.3
$2X10^4$	23.3	44.4	26.5	34.5
$2X10^5$	20.0	43.1	23.2	35.5
$2X10^6$	16.8	43.5	20.1	38.9

Conclusions: The Bruker real-time PCR assay for the detection of Azole Resistance in *Aspergillus*-positive samples shows excellent analytical sensitivity at low sample concentrations and is being further developed to produce a CE-IVD kit to complement the Fungiplex Aspergillus product. Identification of Azole Resistance in patients will provide evidence for appropriate antifungal therapy in a timely manner.