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AspID - a multiplex qPCR kit for the detection of clinically relevant Aspergillus species

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Background: Real-time quantitative PCR (qPCR) has become the most widely used molecular technology for diagnostic applications designed to detect and quantify pathogens. *Aspergillus*-specific qPCR assays have been proposed as alternatives to conventional diagnostic procedures for Invasive Aspergillosis, where early diagnosis and treatment are critical. Our objective was to develop a qPCR kit designed to detect clinically relevant *Aspergillus* species whilst simultaneously capable of identifying *Aspergillus terreus,* an amphotericin-resistant fungus associated with a high mortality rate.

Material/methods: Assay design, optimisation and validation were performed in strict compliance with the MIQE guidelines (1). Sequences of target organisms were aligned in CLC Sequence Viewer to identify suitable target sequences. Primer and probe sequences were designed by Beacon Designer. *In silico* analysis was performed using nucleotide BLAST and the target secondary structure/template accessibility was assessed using MFOLD. SYBR Green chemistry and melt curve analysis were used to determine optimal annealing temperatures and optimal primer/probe concentrations. Pan-*Aspergillus* (FAM) and *A. terreus*-specific (HEX) hydrolysis probe assays were combined with our own internal extraction control assay (ROX) and optimal conditions were established to create *Asp*ID. The assay was extensively validated using DNA extracts from fungal cultures, clinical bronchial washes and serum samples, AsTeC Consortium *Aspergillus* calibrator material (2) and the EAPCRI DNA panel 2013 (3).

Results: Under optimal PCR conditions the primers in OLM's *Asp*ID kits result in amplification efficiencies of >90%. The test has a broad dynamic range of at least six orders of magnitude and can detect down to around 10 copies of target template. Since *Asp*ID targets a region of DNA that has numerous repeats, this is the equivalent of less than one fungal genome. *Asp*ID detects all targets designated 'Essential' in the EAPCRI 2013 panel.

Conclusions: Conclusions: The *Asp*ID multiplex qPCR test kit sensitively and specifically detects genomic DNA of clinically relevant *Aspergillus* species, with simultaneous identification of *Aspergillus terreus*, which provides clinically useful information due to the latter's intrinsic resistance to Amphotericin B.

1. Bustin et al. Clin Chem 2009;55(4):611-22. Epub 2009/02/28

2. Lyon et al. J Clin Micro 2013;51(7):2403-5. Epub 2013/04/26

3. Morton et al. Med Mycol (2016) doi: 10.1093/mmy/myw093