*Asp*ID - A Multiplex qPCR kit for the detection of clinically relevant *Aspergillus* species

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Introduction

•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 *Aspergillus* species whilst simultaneously capable of identifying *Aspergillus terreus.*
•*Asp*ID is an optimised triplex qPCR assay that detects clinically relevant *Aspergillus* species (FAM), identifies specifically *A. terreus* (HEX), an amphotericin-resistant fungus associated with a high mortality rate and incorporates an internal extraction control assay (ROX)

Materials/Methods

Assay design, optimisation and validation were performed in strict compliance with the MIQE guidelines (1). Genomic sequences of target organisms were aligned in CLC Sequence Viewer to identify suitable assay sites. Primer and probe sequences were designed using Beacon Designer software. *In silico* analyses were performed using primer BLAST and nucleotide BLAST, and PCR amplicon secondary structure/template accessibility were assessed using MFOLD. SYBR Green chemistry and melt curve analyses were used to determine optimal annealing temperatures and primer/probe concentrations. 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, kindly provided by Dr Lewis White (3).

References:
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

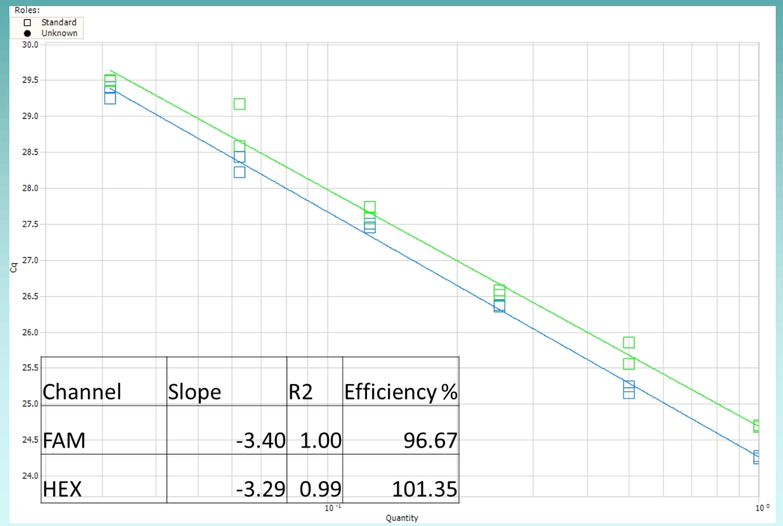

Figure 1. Standard curves generated from *Asp*ID qPCR run on a BioRad CFX96 platform. Blue plot = FAM, Green plot = HEX.

				AspID test results	
Sample Name	Sample	Concentration (fg/µl)	Designation	Assay Name	Result
EAPCRI 1	*A. fumigatus*	150	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative
EAPCRI 3	*A.niger*	150	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative
EAPCRI 7	*A.flavus*	77	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative
EAPCRI 9	*A. fumigatus*	1.5	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative
EAPCRI 11	*A.terreus*	170	Essential	*Pan Aspergillus*	Positive
				A.terreus	Positive
EAPCRI 12	*A.versicolor*	70	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative
EAPCRI 17	*A.lentulus*	300	Essential	*Pan Aspergillus*	Positive
				A.terreus	Negative

Table 1. Results of AspID kit evaluation using the EAPCRI 2013 DNA specificity test panel.

Results

Analytical Specificity:

•*Asp*ID detects *A. fumigatus, A. flavus, A. nidulans* and *A. niger* DNA in the FAM channel only and *A. terreus* DNA in both the FAM and HEX channels.

•*Candida, Absidia, Scedosporium, Fusarium, Rhizopus* or human DNA is not amplified

•*Penicillium* is detected in the FAM channel, but is not detected by the *A. terreus*-specific assay.

Analytical Sensitivity:

•Sensitive to <10 copies of *Aspergillus* target template (equivalent to <1 fungal genome), with a broad dynamic detection range of at least six orders of magnitude.

•Under optimal PCR conditions the primers in *Asp*ID result in amplification efficiencies of >90% (example graph shown in Figure 1).

•*Asp*ID was validated using the EAPCRI 2013 DNA panel and achieved all designated 'Essential' detection requirements (see Table 1).

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.*

*Asp*ID has been commercialised by OLM Diagnostics, to allow direct detection on clinical nucleic acid extracts, with results within 90 minutes of nucleic acid extraction. The kit includes an Internal extraction control (IEC) and Positive control. Further information is available from the OLM Diagnostics booth (booth 18).

Disclosures: Gemma Johnson is employed as Scientific Director at OLM Diagnostics. OLM Diagnostics funded this development project.