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Poster 37

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Cross-reactivity of an *Aspergillus* spp. quantitative PCR based on an 18S rRNA probe: experience of the Mycology Reference Centre Manchester

Introduction

MRCM uses qPCR to detect and quantify *Aspergillus* species in respiratory samples from patients with IA, CPA or ABPA, in order to guide systemic antifungal therapy. For CPA patients on long-term azole therapy at the National Aspergillosis Centre, it is used to monitor and detect treatment failure[1].

The *Aspergillus* spp. ELITe MGB® kit includes a minor-groove binder that allows the probe to be relatively short. As it is based on a consensus 18S rDNA sequence, we wondered whether this could lead to false positives through cross-reactivity with moulds such as *Penicillium* spp. whose 18S rDNA are very similar.

Aspergillus spp. ELITe MGB® kit

- CE-IVD validated
- Beacon probe against 18S rDNA
- FAM fluorophore
- 97.8% clinical specificity
- *Aspergillus* **fumigatus**, *niger*, *nidulans*, *terreus*, *flavus*, *versicolor*, *glaucus*

Methods

Spore DNA preparation
- 20,000 spores suspended in PBS with 0.05% Tween80
- Quantified using a Neubauer haemocytometer
- Pre-treated with ELITech EXTRABlood prelysis kit
- Extracted with ELITe STAR 200 platform

qPCR
- ELITech ELITe MGB® qPCR Master Mix, CTR-CPE internal control plasmid and Asp ELITe Standards; 3 biological replicates
- Melt curves generated using ABI 7500 Fast Dx system

IA invasive aspergillosis; CPA chronic pulmonary aspergillosis; ABPA allergic bronchopulmonary aspergillosis

[1]Moazam *et al* (2020) *Mycoses* 63(4):376-381
[2]Vergidis *et al* (2020) *Clin Microbiol Infect* 26(7):935-940

Results

https://mrcm.org.uk/

qPCRs of species previously isolated in clinical samples by our laboratory

- All 5 control *Aspergillus* species gave a strong positive result (5,800-88,700 copies). Melting temperatures were similar among *A. fumigatus*, *A. flavus* and *A. sydowii* (68°C), with a slightly higher temperature (69°C) for *A. niger* and *A. terreus*.
- Strong positive results were also produced by *Penicillium chrysogenum* and *Paecilomyces variotii*, weal positive by *Penicillium rubens*, all with fewer copies (all <3,000). Their melting temperatures (67-68°C) were very similar to *Aspergillus* controls.
- *Rasamsonia piperina* also gave a strong positive result but with a distinctly lower melt temperature (64°C). The melt temperatures of other species were significantly lower than that for *Aspergillus* and the 3 non-related species were qPCR negative.

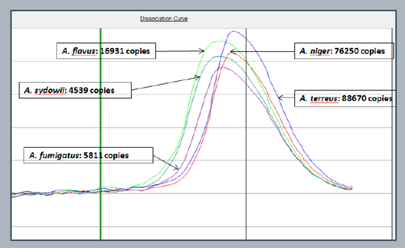
	Species	PCR result*	Copies	Melting temp (°C)
Aspergillus controls	*Aspergillus fumigatus*	STRONG POSITIVE	5,811	68.0
	Aspergillus flavus	STRONG POSITIVE	16,931	68.0
	Aspergillus niger	STRONG POSITIVE	76,250	69.1
	Aspergillus terreus	STRONG POSITIVE	88,670	69.1
	Aspergillus sydowii	STRONG POSITIVE	4,539	68.0
Clinical isolates from samples sent to MRCM	*Penicillium chrysogenum*	STRONG POSITIVE	2406	68.1
	Penicillium rubens	WEAK POSITIVE	1209	66.9
	Penicillium indicum	Negative	18	65.6
	Penicillium amphipolaria	Negative	10	54.4
	Talaromyces thermophiles	Negative	-	-
	Talaromyces pinophilus	Negative	-	-
	Talaromyces piceae	Negative	20	54.2
	Talaromyces columbinus	Negative	-	-
	Talaromyces alboverticulus	Negative	-	-
	Thermomyces dupontii	Negative	-	-
	Thermomyces lanuginosus	Negative	45	58.5
	Hamigera inflata	Negative	191	67.8
	Rasamsonia argillacea	Negative	40	64.6
	Rasamsonia piperina	STRONG POSITIVE	6543	64.1
	Paecilomyces variotii	STRONG POSITIVE	1774	68.1
Distantly-related outliers	*Scedosporium boydii*	Negative	11	-
	Fusarium oxysporum	Negative	4	-
	Trichophyton rubrum	Negative	5	-

***** >1430 copies = strong positive; 1430-1170 copies = weak positive; <1170 copies = negative

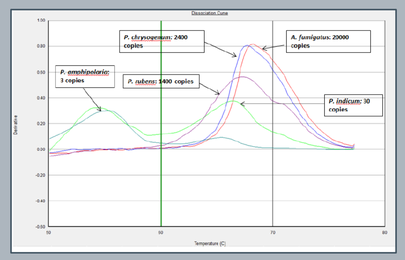
Dissociation curve (melt curve) analysis

Melt curves were obtained after the qPCR run, by gradually raising the temperature until the DNA strands separate. The curve produced for different PCR products (amplicons) within a mixture may be different due to differences in nucleotide sequence, GC content and length.

We examined melt curve data from 2,459 qPCR-positive patient samples and found that 56 (2.3%) may represent non-*Aspergillus* species.



Melt curve analysis could not distinguish between *A. fumigatus* (pink), *A. flavus* (green) and *A. sydowii* (teal) effectively, but further analysis may be able to resolve *A. niger* (red) and *A. terreus* (purple)



Melt curve analysis could not distinguish between *A. fumigatus* (red) and *P. chrysogenum* (purple) but may be used to distinguish *P. rubens* (pink) and *P. indicum* (green).

Conclusions

- *Penicillium chrysogenum*, *P. rubens, Paecilomyces variotii* and *Rasamsonia piperina* were qPCR-positive; melt curves could be used to distinguish some of these species from *Aspergillus*. While care is taken when interpreting the results of this test in a clinical context, the frequency of these events was minimal and supported by positive high-volume culture[2]
- Approximately 2.3% of patient positive qPCRs with unusual melt curves may have been false positives caused by cross-reactivity with other species
- We are currently investigating the use of an *A. fumigatus*-specific probe to complement our use of this qPCR.