Evaluation of a new multiplex PCR kit for detection of clinically relevant *Aspergillus* species in BAL fluid

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Background

*Asp*ID (OLM innovations, Newcastle, UK) is a new assay based on multiplex real-time PCR for detection of clinically relevant *Aspergillus* species. The diagnostic performance of this assay was evaluated within the QCMD 2016 *Aspergillus* spp. DNA EQA Programme and in bronchoalveolar lavage fluid (BALF) specimens from patients at risk of developing invasive pulmonary aspergillosis (IPA).

Materials and Methods

The accuracy of the *Asp*ID was determined utilizing the Quality Control for Molecular Diagnostics (QCMD) 2016 *Aspergillus spp.* DNA EQA Programme. The panel consisted of nine members including *Aspergillus conidia* or *Aspergillus fumigatus* and negatives.

Thirty-six BALF specimens obtained from 18 patients with IPA and 18 with no evidence for IPA were studied (**Table 1**). Patients were classified as having IPA when BALF galactomannan (GM) determination yielded an optical density index (ODI) >3.0 and patients had clinical and radiological findings compatible with IPA. Those without IPA had a BALF GM ODI <0.5 and no clinical/radiological findings compatible with IPA. Patients with and without IPA were matched 1:1 regarding underlying diseases and ICU admission. For detection of *Aspergillus* DNA, BALFs were extracted using the specific B protocol of the NucliSENS easyMAG instrument (bioMérieux, Marcy-l´Etoile, France) with an input volume of 400 µl and an elution volume of 40 µl. After the lysis step, 4 µl of internal extraction control included in the *Asp*ID assay was added. Amplification and detection were performed on the LC 480 II instrument (Roche Diagnostics, Rotkreuz, Switzerland).

Table 1 Characteristics of patients with IPA and without IPA

	Patients with IPA (n=18)	Patients without IPA (n=18)
Mean age (age range)	64.5 yrs (48 – 84)	66.5 yrs (25 – 81)
Female	10	9
Risk factors for IPA*		
Hematological malignancy	3	3
ICU admission	12	13
Solid tumor	4	4
Autoimmune disease	2	2
Mean BALF GM levels (range)	6.9 ODI (3.2 – 25.0)	0.1 ODI (0.1 – 0.3)
Asp. positive BALF culture	7	0

*A patient may have more than one underlying risk factor
Abbreviation: Asp. = Aspergillus

Results

When accuracy was determined, five out of six *Aspergillus fumigatus* positive samples were identified as positive; however, the assay was not able to detect one panel member correctly that contained *Aspergillus fumigatus* DNA (**Table 2**). All *Aspergillus* negative samples were correctly identified as negative.

Table 2 Accuracy testing utilizing the Quality Control for Molecular Diagnostics (QCMD) 2016 *Aspergillus spp*. DNA EQA Programme

Vial no.	Sample content	Matrix	Result expected	Result obtained
1	Negative	TE buffer	Negative	Negative
2	*Asp. fum*. DNA	TE buffer	Positive	Positive
3	*Asp. fum*. DNA	TE buffer	Positive	Positive
4	*Asp. fum*. DNA	TE buffer	Positive	Negative
5	*Asp. fum.*	Synth. sputum	Positive	Positive
6	Negative	Synth. sputum	Negative	Negative
7	*Asp. fum.*	Synth. sputum	Positive	Positive
8	Negative	Plasma	Negative	Negative
9	*Asp. fum.*	Plasma	Positive	Positive

Abbreviations: Asp. = Aspergillus; fum. = fumigatus; Synth. = Synthetic

With the new *Asp*ID, 20 BALF samples were found to be positive and 14 negative (**Fig. 1**). Two samples showed inhibition and were excluded from analysis. When *Asp*ID results were compared to those obtained from GM determination, 29 were found to be concordant and 5 discordant (four *Asp*ID-positives in patients without IPA and one *Asp*ID-negative in a patient with IPA). Sensitivity, specificity, positive- and negative likelihood ratio for *Asp*ID including 95% confidence interval (CI) were 94.1% (95%CI 73.3 – 99.9), 76.5% (95% CI 50.1 – 93.2), 4 (95% CI 1.7 – 9.5) and 0.1 (95% CI 0.01 – 0.5) respectively.

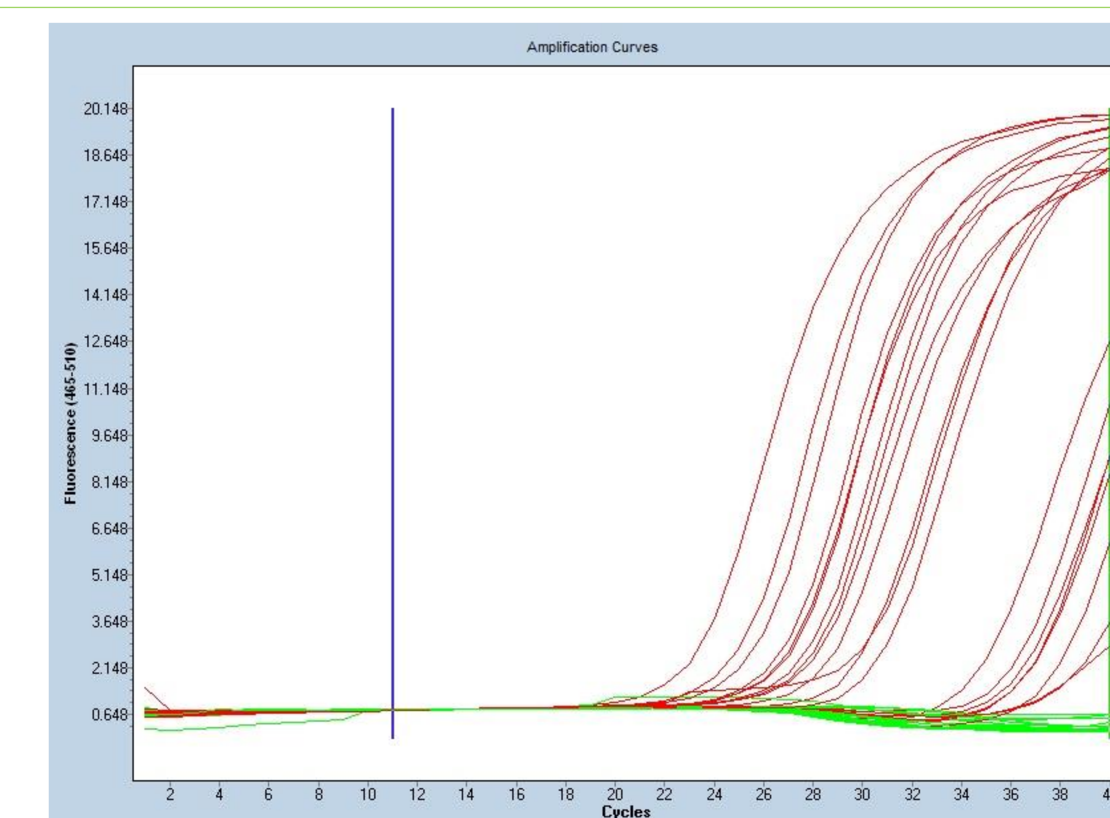

Fig. 1 Fluorescence curves obtained from 20 BALF samples that were found to be positive for *Aspergillus spp.* DNA (red) and 14 BALF samples that were found to be negative (green)

Conclusions

Detection of clinically relevant *Aspergillus spp*. in BALF specimens with *Asp*ID PCR reagents seems to be a promising diagnostic approach in patients at risk for IPA. It may allow early diagnosis and rapid initiation of anti-mold therapy. Four results may have been false positive. This may be due to contamination during bronchoscopy or during the laboratory workflow, possibly through *Aspergillus* contamination in the airways.

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