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Detection of clinically relevant cryptic Terrei species using AspID Multiplex qPCR kit

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Background: Specific identification of *Aspergillus terreus* is clinically useful, as invasive infections caused by *A. terreus* are frequently associated with high resistance to the antifungal drug amphotericin B and with poor survival. Sequencing has recently identified several new species within the section *Terrei*, most of which have shown decreased in vitro susceptibilities to amphotericin B, and whilst morphologically similar to, are genetically distinct from *A. terreus* isolates. Hence it is therapeutically beneficial for assays targeting *A. terreus* detection to be able to detect these cryptic species of the section *Terrei*. Our objective was to determine the detection capability of the *A. terreus*-specific assay of OLM Diagnostics' *Asp*ID multiplex qPCR kit.

Material/methods: A panel of 23 *A. terrei* DNA eluates, identified according to the latest taxonomic standards that represent the whole section were prepared. This panel included 7 *A. terreus*, 5 *A. citrinoterreus*, 5 *A. alabamensis*, 4 *A. hortai*, 1 *A. floccosus* and 1 *A. neoafricanus* DNA sample. DNA quality and concentration was measured for each sample using a NanoDrop 2000 and adjusted to 60pg/µL. Seven point standard curves were constructed for individual *Terrei* targets by five-fold serial dilutings from 60pg/µL to 3.84fg/µL. Each standard point was assayed in triplicate using the *Asp*ID qPCR assay, with positive and negative controls, as recommended by the manufacturer. Results were used to determine the efficiencies and limits of detection for each cryptic *Terrei* species.

Results: *A. terreus, A. citrinoterreus, A. alabamensis* and A. *hortai* DNA eluates were detected in both the pan-*Aspergillus* (FAM) and *A. terreus*-specific (HEX) channels, with efficiency range 99-116% (R^2 0.98-0.99) and 100-118% (R^2 0.92-0.99) respectively. All of these species were detected at the lowest standard concentration of 3.84fg/µL. *A. neoafricanus* was detected in both channels (FAM efficiency 99%, HEX 95%), but limit of detection was 19.2fg/µL. *A. floccosus* was only detected in the pan-*Aspergillus* channel, with a detection limit of 19.2fg/µL.

Conclusions: OLM Diagnostics' *Asp*ID kit can be used to sensitively and efficiently identify the cryptic species *A. citrinoterreus, A. alabamensis*, *A*. *hortai* and *A. neoafricanus* as being from the section *Terrei*. This specific detection of both *A. terreus* and closely related cryptic species will benefit therapeutic decision making, by confirming the presence of an infectious species with highly documented amphotericin B resistance. Although *Asp*ID was not able to identify the cryptic species *A. floccosus* beyond its genus *Aspergillus*, due to mismatches in the forward primer binding region, the majority of new species from the *Aspergillus* section *Terrei* were identified as *A. terreus*.