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A Novel Multiplex PCR Assay with Melting Curve Analysis for Rapid Detection of *Aspergillus* Species and Azole Resistance

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BACKGROUND

Aspergillus spp. are filamentous, environmental fungi that cause a wide spectrum of infections in humans, including hypersensitivity reactions, chronic pulmonary infections, and acute life-threatening infections. The diagnosis of aspergillosis remains difficult, and the rapid emergence of azole resistance in *A. fumigatus* is worrisome. The novel multiplex PCR assay with melting curve analysis provides a solution in this matter. The aim of this study was to validate the MycoMDx *Aspergillus* PCR Assay Kit and *A. fumigatus* resistance multiplex assay Kit produced by Dynamiker Biotechnology (Tianjin) Co., Ltd., and to evaluate its performance on clinical samples for the detection of four common *Aspergillus* species (*A. fumigatus/A. flavus/A. terreus/A. niger*) and three resistance-associated mutations (L98H/Y121F/T289A) in the Cyp51A gene. (Figure 1).

METHOD

The diagnostic criteria of this study were based on the 2019 EORTC/MSGERC Definitions of invasive fungal diseases. Thirty-two patients with proven/probable aspergillosis and fifty non-aspergillosis patients were enrolled and detected for Aspergillus PCR and azole resistance.

RESULT

The results showed that the sensitivity and specificity of *Aspergillus* PCR in bronchoalveolar lavage fluid (BALF) were 93.75% (30/32) and 98.00% (49/50). A total of four common clinical *Aspergillus* species were detected, among which *A. fumigatus* appeared the most frequently, followed by *A. flavus*, *A. terreus*, *A. niger*. In addition, resistance-associated mutations were detected in 6 BALF samples by *A. fumigatus* resistance multiplex assay, including 3 samples containing L98H mutation, 2 samples containing Y121F mutation, 1 sample containing T289A mutation.

CONCLUSION

The results of this study indicate that the novel multiplex PCR assay with melting curve analysis can identify four medically important *Aspergillus* species DNA and detect presence of the L98H/Y121F/T289A mutation.

KEY WORDS

Multiplex PCR; Azole Resistance; *Aspergillus* species

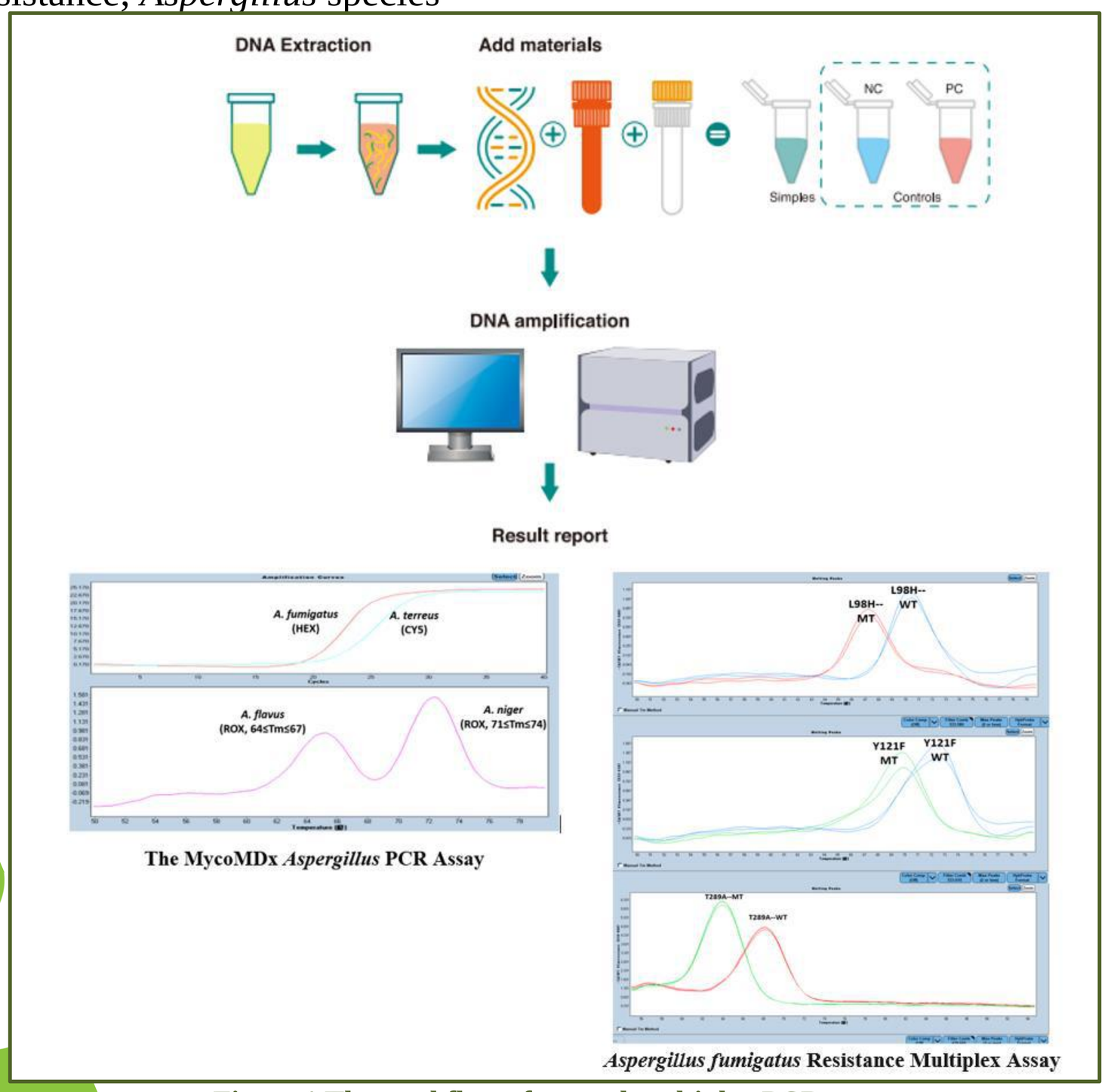

Figure 1 The workflow of a novel multiplex PCR assay

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