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# A novel multiplex PCR assay with melting curve analysis for rapid detection of Azole-Resistant *Aspergillus fumigatus*

**06. Fungal infection & disease**

6b. Diagnostic mycology (incl molecular)


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**Background** Since itraconazole resistant Aspergillus fumigatus was first reported in the United States in 1997, the detection rate of triazole resistant A. fumigatus was as high as 20%~30% worldwide, and the mortality rate of Invasive Aspergillosis (IA) caused by resistant strains was 88%~100%. Due to the lack of effective means of early diagnosis, clinicians often take preemptive treatment or experiential treatment, resulting in the irrational use of antifungal drugs, aggravating the patient's condition. There is strong evidence that environmental exposure to azole fungicides is driving the emergence of TR34/L98H and TR46/Y121F/T289A resistance mechanisms. Conventional culture remains insensitive but is required for in vitro susceptibility testing to detect azole resistance. Direct detection of resistance mutation by polymerase chain reaction (PCR) from clinical specimens can identify resistance. The combined use of fluorescence color and melting temperature (Tm) as a virtual 2D label that enables homogenous detection of one order of magnitude more targets than current strategies on real-time PCR platform. The novel multiplex PCR assay with melting curve analysis provides a solution in this matter, because it can be used directly on clinical sample (serum, BAL) to identify the most prevalent resistance mechanisms (Dynamiker Biotechnology (Tianjin) Co., Ltd.). (Figure1 A).

**Methods** In total, 5 wide type of A. fumigatus, 4 clinical isolates containing TR34 mutation, 3 clinical isolates containing TR46 mutation, 3 clinical isolates containing L98H/Y121F/T289A mutation were detected by A. fumigatus resistance multiplex assay. At the same time, the data were confirmed by sequencing.

**Results** The results obtained by novel multiplex PCR assay with melting curve analysis for rapid detection of Azole-Resistant A. fumigatus were consistent with the sequencing results (Figure1 B).

**Conclusions** The novel multiplex PCR assay with melting curve analysis for rapid detection of Azole-Resistant A. fumigatus could be used as an adjunct diagnostic for IA.

Figure1 Aspergillus resistance multiplex assay

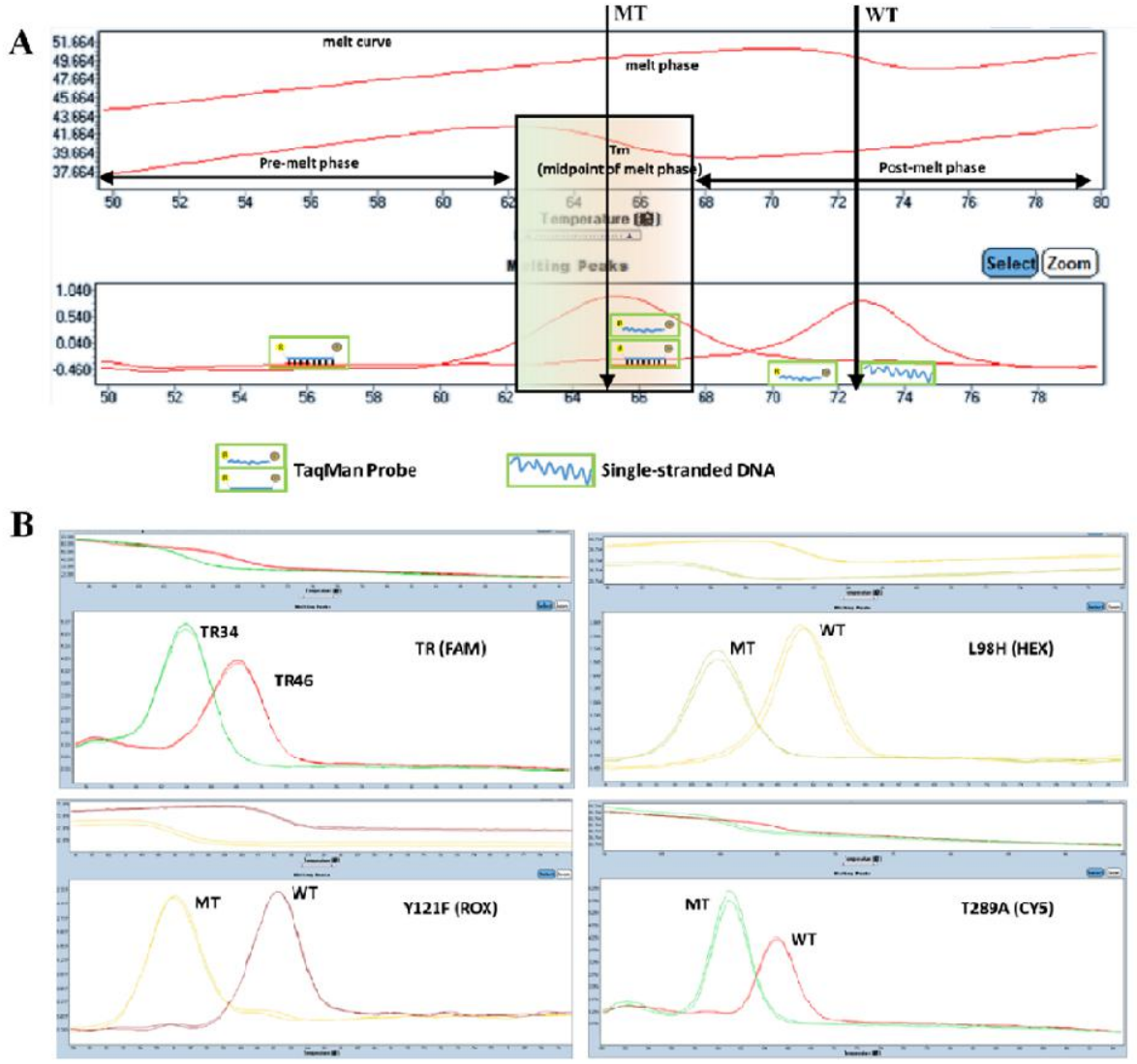


**Figure1 *A. fumigatus* resistance multiplex assay. A.Schematic diagram of fluorescence melting curve analysis with molecular beacons; B. Results of detecting 5 target genes with *A. fumigatus* resistance multiplex assay.**

**Keyword 1**
A. fumigatus
**Keyword 2**
Azole resistance
**Keyword 3**
multiplex PCR

**Conflicts of interest**

**Do you have any conflicts of interest to declare?**
I have no potential conflict of interest to report