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A novel multiplex PCR assay for the identification of clinically relevant *Aspergillus* species

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Abstract:
BackgroundWith the increase of preventive antifungal therapy, the distribution of pathogenic bacteria has changed greatly. *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger* have become the main clinically relevant *Aspergillus* species. To realize the early diagnosis of *Aspergillus* infection, the *Aspergillus* PCR Assay Kit based on the fluorescence PCR method was developed. According to the characteristics of the TaqMan fluorescent probe and fusion curve analysis, the kit can simultaneously identify the detection genes of *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger* by a single PCR reaction. (Figure1)**Methods**We evaluated the detection performance of the *Aspergillus* PCR Assay Kit. Forty samples with definite clinical diagnosis were selected to verify the sensitivity and specificity of the kit using tissue culture method as the gold standard; DNA of 16 strains of *Aspergillus* and other respiratory pathogens were detected to verify the cross-reactivity; Thirty-two tests were carried out on the preservation solutions of four *Aspergillus* species, to verify the degree of precision; The spore suspensions of four *Aspergillus* species after gradient dilution were repeated 20 times to determine the final limit of detection (LoD).**Result**The results showed that the sensitivity of the kit was 100% (20/20), the specificity was 95% (19/20), and the kit is not cross-reactive to strains other than the four *Aspergillus* species. The degree of precision was 96.88% (31/32) and the final limit of detection was 1 copies/μl. All the verification parameters reached acceptable standards and the detection results were reliable, which could meet the needs of clinical diagnosis.**Conclusions**The Dynamiker *Aspergillus* PCR Assay Kit can achieve rapid and accurate identification of clinically relevant *Aspergillus* species in clinical samples (serum and alveolar lavage fluid) in vitro, and can be used for auxiliary diagnosis of deep *Aspergillus* infection.

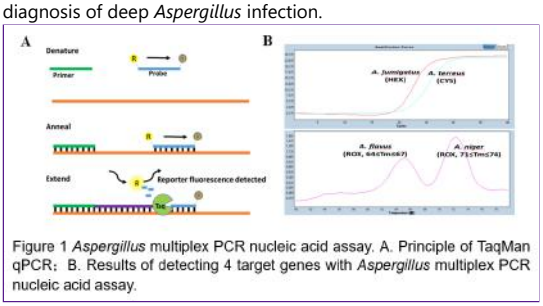

Figure 1 *Aspergillus* multiplex PCR nucleic acid assay. A. Principle of TaqMan qPCR; B. Results of detecting 4 target genes with *Aspergillus* multiplex PCR nucleic acid assay.

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