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Oral Session

Advances in molecular mycology: notes from the bench

A NEW DIAGNOSTIC ASSAY FOR THE DETECTION OF *ASPERGILLUS FUMIGATUS* AND ITS RESISTANCE PATTERN BY REAL-TIME PCR

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Objectives

Aspergillus fumigatus is the most common cause of invasive pulmonary aspergillosis (IPA), which is prevalent in immune-compromised patients. Current diagnostic methods generally rely on galactomannan and/or 1,3-beta-glucan in combination with culture, which is negative in 50-80% of the cases. Mortality rates up to 90% are observed in these patients, in part due to the increased Voriconazole resistance (now 10% in the Netherlands). We developed and validated a commercial real-time PCR assay (CE-IVD) for fast and accurate detection of *Aspergillus fumigatus*, and its resistance pattern, in BronchoAlveolar Lavage (BAL) from patients suspected of invasive fungal infection.

Methods

We developed 3 quadruplex PCR assays that give comprehensive results within 2,5 hours. The first assay can detect *Aspergillus spp* and specifically *A.fumigatus* and *A.terreus*. The second assay detects and differentiates the resistance markers L98, tandem repeat 34 (TR34) and 46 (TR46). The third assay detects and differentiates resistance markers G54, G138 and M220. The assays make use of self-quenching probes by means of quantitative amplification, and the differentiation of wildtype from mutant is accomplished by melting curve analysis using target specific probes. We also developed a DNA extraction/concentration method from high volumes of BAL material, to achieve an increased sensitivity with the diagnostic assays.

Results

The quadruplex assays were validated according to MIQE and CE-IVD guidelines. The *Aspergillus spp.* probe in the first assay can detect *A.fumigatus*, *A.terreus*, *A.niger* and *A.flavus* strains. Limit Of Detection (LoD) experiments were performed using quantified reference strains resulting in 0.5-1 conidia by the multicopy gene. All resistance assays resulted in a LOD of 6-12 conidia. A 90-100% score was obtained with Quality control for molecular diagnostics (QCMDs) samples of 2011, 2012 and 2013 by using this assay.

The second quadruplex assay was extensively tested on 141 positive *Aspergillus* cultures from IPA patients resulting in the detection of 17 TR34/L98 mutant strains. The wildtypes and resistant strains were successfully differentiated by melting peaks at different temperatures. Other mutations were not observed in the selected patient group but were tested using proven cultures of those specific *Aspergillus* mutants. A total of 40 BAL samples were tested with the assays, showing a higher sensitivity than galactomannan and culture. The discrepancies between tests were confirmed with sequencing.

Conclusion

We developed and validated diagnostic PCR assays that are able to detect and differentiate *Aspergillus* species directly from BAL samples. The PCR assay is further able to detect and differentiate wildtype and mutant *A.fumigatus* strains, as well as simultaneously determine the most prevalent resistance patterns within 2,5 hours, which is not possible with culture alone.