eP390
ePoster Viewing
Highlights from molecular mycology

DEVELOPMENT OF A NEW COMMERCIAL QPCR ASSAY TO DETECT AND DIFFERENTIATE DERMATOPHYTE INFECTIONS OF THE SKIN, NAILS AND HAIR.

G. Dingemans[1], M. Van den Bosch[1], M.P. Hayette[2], S. Goethel[3], V. Rusu[3], R. Sacheli[2], J. Meis[4], G. Gaajetaan[1], G. Simons[1]

[1]R&D, PathoNostics, Maastricht, Netherlands ; [2]Microbiology, University Hospital Liége (CHU), Liége, Belgium ; [3]R&D, MagnaMedics GmbH, Aachen, Germany ; [4]Mycology, Canisius Wilhelmina Ziekenhuis (CWZ), Nijmegen, Netherlands

Objectives

Superficial dermatophytosis is the most common fungal infection in humans. Dermatophytes are keratinophilic fungi which are able to infect keratinized tissue. Diagnosis of dermatophytosis is based on microscopic observation of fungal structures in KOH treated skin scales plus culturing and identification of the causative species. However, direct microscopy lacks specificity and culturing is time-consuming because it requires generally 2-4 weeks. Therefore, we have developed 3 quadruplex qPCR assays for the fast detection of the clinical most relevant pathogenic dermatophytes. In order to offer a complete diagnostic system, we also developed a fully integrated extraction method which dissolves keratin tissue and efficiently extracts the DNA of the fungus.

Method

The diagnostic assays make use of self-quenching probes by means of quantitative amplification and 2 to 3 pathogens can be distinguished with the same probe by melting curve analysis. Three different assays are developed based on the type of material; 1) nail-, 2) hair- and 3) skin-assays. The nail assays can detect and differentiate T.rubrum (probe 1), T.mentagrophytes/T.interdigitale (probe 2) and C.albicans from C.parapsilosis (probe 3). The hair assay can detect and differentiate M.canis from M.audouinii (probe 1), T.violaceum from T.soudanense (probe 2) and T.mentagrophytes from T.tonsurans (probe 3). The skin probe can detect and differentiate C.albicans from C.parapsilosis (probe 1), E.floccosum (probe 2), T.rubrum (probe 3) and T.mentagrophytes/interdigitale (probe 4). Common fluorescent labels were used in these assays and one probe was designed for an internal amplification control.

Results

Assays for dermatophyte skin and nail infections were verified according to MIQE and CE-IVD guidelines and showed very good sensitivity and specificity. Well characterized samples were used from IHEM and ATCC for the verification of the assays, and the LoDs of the assays ranging from 10-15 copies/reaction. A set of 50 nail samples were used for an initial validation of the nail assay. Results were compared with microscopy and culture which showed a good correlation. ITS sequencing was used to test discrepancies In total, almost 40% (20) nails were found with onychomycosis. Moreover, the nail assay was able to detect an additional 16% (8) of pathogenic dermatophytes in culture negative samples.

Conclusion

The diagnostic nail assay was able to detect the most prevalent pathogenic dermatophytes species in clinical specimens and proved to be more sensitive and specific than culture and direct microscopy. Other assays (hair and skin) are currently in the validation phase. A new (integrated) extraction procedure was proven to work efficient in diagnostics and the total analysis time was fast (<3,5 hours) which enables the treating physician to start a dedicated treatment within the same day.