PATHONOSTICS

Development of a new commercial qPCR assay to detect and differentiate dermatophyte infections of the skin, nails and hair.

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Objective

The objective of the Fast Fungal Diagnostics (FFD) project is to develop 3 multiplex qPCR assays for the fast detection of the clinical most relevant pathogenic dermatophytes in skin, nails and hair samples. In order to offer a complete diagnostic system, a fully integrated DNA extraction method was developed as well.

Background

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.

Materials and Methods

The diagnostic assays enables fast (<3,5h) quantitative amplification and 2 to 3 pathogens can be distinguished with the same probe by melting curve analysis of self-quenching probes. The ITS region is used as target for the differentiation of most dermatophytes. In total 3 different assays are developed based on the type of material; 1) nail-, 2) hair- and 3) skin-assays. An easy to perform DNA extraction method is set-up, consisting of: I) solubilisation of the material (nails), II) lysis and III) isolation and purification using a combination of glass-beads and magnetic beads.

This method is able to dissolve keratin tissue and efficiently extracts the DNA of the fungus. In addition, an Internal Control (IC) is added to the clinical material to monitor all steps of the reaction and to control for inhibition or manual errors.

Composition qPCR assays

1) Nail multiplex	2) Hair multiplex	3) Skin multiplex	
C. albicans *C. parapsilosis*	*M. canis* *M. audouinni* *M. ferrugineum*	*M. canis* *E. floccosum*	GREEN
T. mentagrophytes var. interdigitale	*T. mentagrophytes* *T. tonsurans*	*T. mentagrophytes* *T. mentagrophytes var. interdigitale*	YELLOW
T. rubrum	*T. soudanense* *T. violaceum*	*T. rubrum*	ORANGE
Internal control	Internal control	Internal control	RED

Melting curves Nail panel

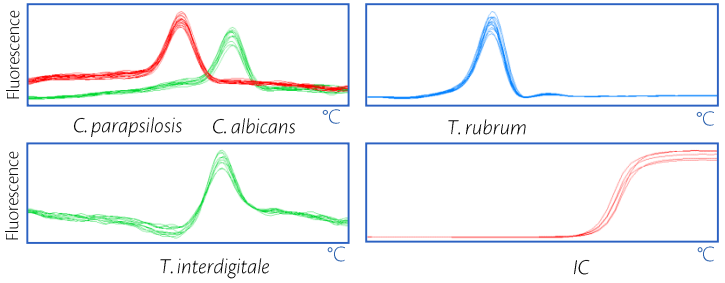

Fig 1: fungal species identified by melting curve analysis obtained with the Nail panel. Analysis was performed on a Rotor-GeneQ (Qiagen). The assay is validated on a LightCycer LC480 (Roche) as well.

Sensitivity qPCR assays

Complex	Tested on # strains	Detection limit / reaction
C. albicans / C. parapsilosis	*15*	*10 copies*
M. canis / M. audouinni / M. ferrugineum	*34*	*5-15 copies*
T. rubrum / T. violaceum / T. soudanense	*53*	*5-15 copies*
T. mentagrophytes var. interdigitale / T. mentagrophytes / T. tonsurans	*31*	*5-15 copies*
E. floccosum	*Not validated yet*	

Results

The 3 dermatophyte assays were able to correctly identify all dermatophyte species and no cross-reactivity was observed. In total 133 fungal strains were tested, of which the identity was confirmed by sequencing and AFLP. Sensitivity of the assays was determined using digital PCR (Biorad) and LoD values were determined as 5-15 copies/reaction. Because the assay is optimized recently, the validation will be conduced in the upcoming months on 122 nails as well as on hair and skin material. Initial experiments on a small set of nails, showed *T.rubrum* in all positive nails.

Conclusion:

A new complete diagnostic system was designed and developed with an integrated extraction procedure and showed good performance with great potential to implement as a fast diagnostic tool for dermatophyte infections.