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Comparison between Real Time PCR and culture analysis to detect dermatophyte infections

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INTRODUCTION AND PURPOSE

Dermatophytosis, caused by *Trichophyton, Microsporum* and *Epidermophyton,* is a fungal infection that affects nails, skin and hair. Conventional diagnostics involves culture and microscopical analysis.
Recently, molecular diagnosis by Real Time PCR has been spreading. This PCR kit allows a rapid and reliable fungal identification of 28 dermatophytes as the same time in a single tube.
The aim of this study is to demonstrate utility and efficacy of rapid molecular diagnosis of dermatophytes that allows a timely treatment

MATERIAL AND METHODS

Trichophyton tonsurans

Epidermophyton floccosum

Candida albicans

***Microsporum* spp.**

- *M. audouinii*
- *M. canis*
- *M. ferrugineum*

***Trichophyton rubrum* complex**

- *T. rubrum*
- *T. balcaneum*
- *T. circonvolutum*
- *T. gourvilii*
- *T. kuryangei*
- *T. soudanense*
- *T. violaceum*
- *T. yaoundei*

EXTRACTION PROTOCOL

- Vigorously vortex UTM-RT containing the nail fragments
- Take 600 UL in Power Beads
- Vortex for 10 minutes at 1200RMP
- Spinning
- Collect the supernatant in a new screew skirt
- Bring to volume of 600 UL with ATL
- Add 50 UL of PK and incubate at 56° for at least one hour
- Proceed with automatic extraction with QIASYMPHONY

***Trichophyton mentagrophytes* complex**

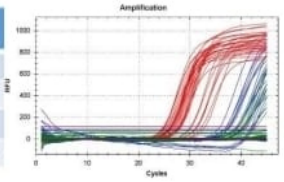
- *T. mentagrophytes*
- *T. benhamiae*
- *T. bullosum*
- *T. concentricum*
- *T. erinacei*
- *T. eriotrephon*
- *T. equinum*
- *T. interdigitale*
- *T. quinckeanum*
- *T. schoenleinii*
- *T. simii*
- *T. tonsurans*
- *T. verrucosum*
- *T. cf.schoenleinii /quinckeanum*

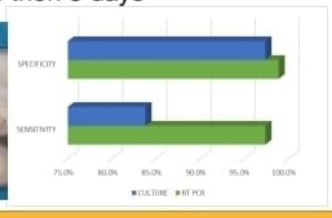
PCR ASSAY The Real Time PCR kit (Novaplex Dermatophyte Assay – Seegene, Republic of Korea) detects 8 substypes of *Tricophyton rubrum* complex (TRC), 14 subtypes of *Trichophyton mentagrophytes* complex (TMC), 3 subtypes of *Microsporum spp* (Mspp), *Trichophyton tonsurans* (TT), *Epidermophyton floccosum* (EF) and *Candida albicans* (CA). For this study we collected cutaneous swabs from 165 patients with signs and symptoms of dermatophytosis. All samples were analized in culture and Real Time PCR.

RESULTS

We analyzed 165 samples , 75 positive for dermatophyte infections, 90 negative. Data analysy showed a PCR sensitivity of 97.4%, while the specificity was 98.9%. In cultural analysis we found a sensitivity of 83.8% and a specificity of 97.4%.
Pcr detects dermatophites in less then 2 hours, culture detects dermatophites in more then 3 days

	POSITIVE 75	NEGATIVE 90
PCR+	73	89
PCR-	2	1
CULTURE+	60	88
CULTURE-	15	2





CONCLUSION

The most detected dermatophytosis pathogens are *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Trichophyton tonsurans*. The comparative analysis between PCR and culture showed an high percentage of agreement about the specificity of two tests, while a low concordance about sensibility.
These data demonstrate the importance of molecular analysis especially for those patients who have recently used topical medications.
In these cases, in fact, bacterial growth in the plate is inhibited while it is possible to detect the presence of dermathophytes DNA via PCR.
Furthermore, the PCR kit allows to identify dermatophytes that have long time of replication and are also difficult to recognize in culture by an unexperted operator.

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