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ePoster Viewing
Highlights from molecular mycology

COMPARATIVE EVALUATION OF DERMATOPHYTE-PCR KIT, WITH CONVENTIONAL METHODS FOR RAPID DETECTION OF DERMATOPHYTES AND IDENTIFICATION OF TRICHOPHYTON RUBRUM IN NAIL SPECIMENS

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Objectives: Onychomycosisis a common and persistent fungal infection caused chiefly by *Trichophyton rubrum* and *Trichophyton interdigitale.* Diagnosis hasrelied on a combination of microscopyand culture based techniques; however, microscopy lack sensitivity, specificity and their growth may take to weeks. A fast and accurate detection and identification of the causal agentin onychomycosis is essential, since requires long-term systemic antifungal treatment. Recently, many PCR assays have been developed to improve onychomycosis diagnosis. In this study, a commercially available multiplex PCRkit, which combines pan-demartophytes PCR with a *T. rubrum*-specific PCR was compared with KOH microscopy and cultureisolation for diagnosis of dermatophytes in nail specimens. Methods: Nail specimens (n=130) were prospectively collected from patients withclinically suspected onychomycosis and were studied by KOH microscopy, cultures and multiplex PCRkit (Dermatophyte PCR kit, Statens Serum Institut, SSI Diagnostica, Denmark) ina mycology reference laboratory. Fungal DNA was easily and rapidly (15 min)extracted using a commercial kit. The multiplex PCR using novel primerstargeting the pan-dermatophyte-specific sequence of the chitin synthase 1 gene(CHS1) for detecting dermatophytes generally and ITS2 (internal transcribed spacer) for detecting *T. rubrum* was performed. The test was completed within 5 h. Results: More samples were positive by PCR (63/130, 49%) than by culture (36/130, 28%). Only2 culture positive specimens (*T. rubrum* n=1, *T. interdigitale* n=1) were not detected by PCR, possibly due to the small quantity of the examined material. *T.rubrum* could be identified in 45% (26/58)of cases in which the culture results were negative. Since the majority of PCRpositive but culture negative samples were positive in microscopic examinations(19/27), the increased sensitivity was not due to contamination (Table 1). From11 specimens diagnosed by conventional methods as non-dermatophyte or *Candida* species, one gave a positivePCR-result (*T. rubrum*). The sensitivity, specificity, positive predictive value, and negative predictive value of the multiplex PCR was 94%, 69%, 54% and 97%, respectively, when confirmed by positive culture. Conclusion: The multiplex-PCR method applied directly on nail specimens is simple, fast and reliable. Therefore, a polyphasic approach to identification thatcombines conventional method (morphologic identification) and multiplex-PCR method will ensure the greatest success in the management of patients with suspected onychomycosis.

Table 1. Microscopy and culture results from nail specimens with suspected onychomycosis in relation to positive or negative PCR results.

	Positive PCR							
	T.rubrum		Dermatophyte		**Negative PCR**		**Total**	
	n	(%)	n	(%)	n	(%)	n	(%)
Microscopy								
Positive	44	(%65.7)	5	(%7.5)	18	(%26.9)	67	(%51.5)
Negative	14	(%22.2)	0	(%0,0)	49	(%77.8)	63	(%48.5)
Culture								
Positive	32	(%55.2)	2	(%5.6)	2	(%5.6)	36	(%27.7)
Negative	26	(%27.7)	3	(%3.2)	65	(%69.1)	94	(%72.3)

% refers to total number of specimens, n=130