Short Communication

Comparison of a new commercial test, Dermatophyte-PCR kit, with conventional methods for rapid detection and identification of *Trichophyton rubrum* in nail specimens

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The performance of a new commercially available duplex PCR, which combines pan-dermatophyte PCR with a *Trichophyton rubrum*-specific PCR, was evaluated. This Dermatophyte PCR kit, which requires one day for laboratory diagnosis, was compared with the conventional methods of microscopy and culture that necessitate up to 4 weeks for final diagnosis of dermatophytosis. We studied 177 nail samples from patients with suspected onychomycosis by fluorescence microscopy (blankophore), cultures and the Dermatophyte PCR kit. More samples were positive by PCR (78/177, 44%) than by culture (59/177, 34%). *T. rubrum* was present in 95% of all culture-positive nail specimens, which was confirmed by PCR in 55/56 specimens. The positive predictive value, negative predictive value, specificity and sensitivity of the duplex PCR was 93%, 87%, 94% and 85%, respectively, when confirmed by positive culture, microscopy or both. Due to its sensitivity, specificity and rapidity, we conclude that this PCR is an attractive method for routine investigation of nail dermatophytosis in a clinical setting.

**Keywords**  Dermatophyte-PCR, onychomycosis, *Trichophyton*

**Introduction**

Onychomycosis, fungal infections of nails, are mainly caused by dermatophytes, although non-dermatophytes such as yeasts and other molds have been implicated as causative agents [1]. The incidence of onychomycosis is increasing and accounts for up to 90% of cases of toenail and at least 50% of fingernail infections [2]. In Europe, *Trichophyton rubrum* is the chief etiologic agent of onychomycosis, followed by *T. mentagrophytes* and *T. interdigitale* [3,4].

The laboratory diagnostic methods for detection of onychomycosis are based on direct microscopic examination of nail specimens and the culture of portions of these specimens [5]. Direct microscopy of nail material is often adequate for the diagnosis of a fungal nail infection, but does not differentiate dermatophytes from other molds involved in these infections [6]. Species identification requires morphological examination of cultured fungal colonies. Since cultivation of fungi is a slow process (up to 4 weeks) and cultures may generate false-negative results in 40% of the cases that are positive by microscopy, more rapid and sensitive methods for fungal species identification of the etiologic agents of onychomycosis are needed [7].

The direct application of PCR-based methods on clinical samples would allow early and specific diagnosis of onychomycosis and the causative agents. The aim of this study was to evaluate a new commercially available Dermatophyte PCR Kit (Statens Serum Institut, Copenhagen, Denmark), and to determine if it was feasible to replace conventional diagnostic methods (culture and microscopy) with the kit for the detection of dermatophytes and *T. rubrum* in nail samples with suspected onychomycosis.
Material and methods

Routine examination of nail samples

A total of 177 nail samples from patients with suspected onychomycosis were received from January to March 2009 for routine examination at the Unit of Medical Mycology at the Department of Clinical Bacteriology, Sahlgrenska University Hospital, Gothenburg. Specimens were evenly divided into three parts upon arrival for direct microscopic examination, culture and PCR studies. The only requirement was that there should be sufficient nail material to be able to run all three diagnostic methods. Nail material was digested in 30% potassium hydroxide directly on a glass slide to create a flat specimen with sufficient transparency to allow for its examination. The samples were stained with blankophore and examined by fluorescence microscope (Zeiss, Carl Zeiss Inc., NY, USA) (×20 objective). In parallel, the second portion of the nail samples were cultured on Mycobiotic agar plates (containing cycloheximide to inhibit growth of molds contaminants) to facilitate growth of dermatophytes. Sabouraud agar and malt agar plates were also employed for cultivation of non-dermatophyte fungal species, e.g., yeasts and other molds. The plates were incubated at 30°C for 3 weeks under controlled humidity. All fungal isolates were identified by observation of macro- and micromorphology.

Dermatophyte PCR

The duplex PCR was run according to the manufacturer’s recommendation for the dual detection of dermatophytes and *T. rubrum* in nail specimens. Briefly, the nail specimens were incubated at 95°C for 10 min in 100 μl of lysis buffer and subsequent addition of 100 μl of neutralizing buffer. PCR was performed in a total volume of 20 μl containing 10 μl PCR Ready Mix, 6 μl of primer mix and 4 μl of DNA template. The primer mix contained two primer pairs directed against genes encoding chitin synthase (chs 1) for detection of dermatophytes in general, and ITS2 (internal transcribed spacer 2) encoding the *T. rubrum*-specific sequence [8]. Dermatophyte and *T. rubrum* genomic DNA provided by the manufacturer served as positive controls. Buffer mix (a mix of lysis buffer and neutralizing buffer at volume ratio 1:1) was used as negative control. A plasmid originating from a microorganism other than *T. rubrum* was provided by the manufacturer as an internal control. This positive control is amplified by the *T. rubrum*-specific primers, resulting in a fragment of 660 bp. The internal control is used to ascertain that inhibitory substances are not present in the test samples which could result in false negative results. It is required that the internal control be positive in all PCR-negative nail specimens. DNA amplification was performed using a DNA Thermal Cycler (Perkin Elmer Cetus, Waltham, Massachusetts, USA). The presence of specific PCR products was examined by staining with ethidium bromide on 2% agarose gel electrophoresis using DNA molecular weight marker (Roche, Mannheim, Germany).

Results

Detection of dermatophytes and *T. rubrum* in nail samples by PCR

Nail samples (*N=177*) were processed using the Dermatophyte-PCR kit. Amplified DNA from four representative nail samples are shown in Fig. 1. According to the manufacturer, *T. rubrum* positive nail specimens will generate a strong band of 203 bp and a weaker band or none at all of 366 bp (Fig. 1, lanes 3+4). This is due to the relatively higher copy number of ITS2 sequences (203 bp band) compared with csh1 sequences (366 bp band).
Comparison of a new dermatophyte PCR with conventional methods

Comparison of detection of dermatophyte and non-dermatophyte fungi from nail samples by conventional diagnostic methods with duplex PCR

Fungal filaments were observed by microscopy in 81 (46%) of the nail specimens. Conventional cultures yielded dermatophytes from 60 of the same nail samples (34%), most of which subsequently identified as *T. rubrum* (*n* = 56). The three other isolates proved to be *T. interdigitale* (*n* = 2) and *T. mentagrophytes* (*n* = 1). Overall, 78/177 (44%) of the samples were positive by PCR (Table 1). Among the 56 specimens which were culture-positive for *T. rubrum*, 55 were confirmed by PCR as *T. rubrum* (Table 1). Out of the four specimens that were pan-dermatophyte positive and *T. rubrum*-negative in the duplex PCR, two of them were identified as *T. interdigitale* by culture, three were positive by microscopy, and one was negative by both microscopy and culture (Table 1). Conversely, three specimens that were positive by culture (*T. rubrum n* = 2, *T. mentagrophytes n* = 1) were negative in the PCR assay (Table 1). As expected, the PCR was negative in 7 (out of 8) cases that were diagnosed as non-dermatophyte species by conventional culture and microscopy. One specimen that was defined as *Chaetomium* species by culture was identified as *T. rubrum* by PCR. However, the initial PCR result may have been correct since when a second specimen was obtained from the patient it was positive for *T. rubrum* by culture.

*Sensitivity, specificity, positive and negative predictive values*

Diagnostic accuracy was assessed by calculating positive predictive value (PPV), negative predictive value (NPV), specificity and sensitivity for the PCR kit. The definition of a gold standard is difficult since direct microscopy examination of nails does not provide genus or species identification. On the other hand, it is known that 40% of microscopy-positive cases were negative by culture. The PPV, NPV, specificity and sensitivity of the PCR test was 93%, 87%, 94% and 85%, respectively when the gold standard for a true positive sample was that it had been positive by direct microscopy, culture or

![Table 1](image)

by both methods. If microscopy alone was the criterion for a ‘true infection’, the PPV, NPV, specificity and sensitivity of the duplex PCR was 89%, 88%, 91% and 85% respectively.

Discussion

In our hands, the detection rate of dermatophytes in nail specimens with suspected dermatophytosis was 44% for the duplex PCR, 46% by microscopy and 34% by culture. Our results are in agreement with those of Brillowska-Dabrowska *et al.* who reported the same rate of PCR positive samples (44%) [6]. On two occasions, the duplex PCR and culture gave divergent results, i.e., *Chaetomium* species by culture, and *T. rubrum* according to the PCR. This finding may reflect overgrowth by the rapidly growing *Chaetomium* mold in a sample from a patient that was doubly infected by *T. rubrum* and *Chaetomium* spp. Alternatively, this finding may also be due to contamination in the lab or DNA contamination of utilities when sampling. In two other samples, *T. rubrum* and *T. mentagrophytes*, respectively, were obtained by culture but the PCR assay remained negative. Since the internal control was positive in these specimens, we could discard the possibility that PCR inhibitory substances were present in the samples. Possible explanations are that the fungal isolates were non-viable or viable but unevenly distributed in the nail specimen. Another possibility was that the strains had mutations in the sites recognized by the hybridizing. We could not investigate this further by DNA sequencing because no more samples remained.

The PCR provides the clinician with a diagnosis within 1–2 days, which is a great benefit. Although the PCR kit is more expensive than the reagents required for the traditional diagnostics, it is less labor-intensive and hence the costs for the laboratory and the patient are comparable. The disadvantages inherent in the PCR kit, inability to detect non-dermatophyte nail pathogens or to distinguish between anthropophilic and zoophilic fungal species are not major
issues. Since onychomycosis is a benign medical condition and nail clippings are easily accessible, a modest rate of false negative specimens is acceptable. To conclude, the duplex PCR will be our diagnostic assay of choice for toe nail onychomycosis. Finally, the possibility that this new method of diagnostics may ‘select’ for strains with mutations in the gene sequences detected by the probes must be kept in mind if significant drops of detection rates (<40%) occur in the future.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


