Five-Hour Diagnosis of Dermatophyte Nail Infections with specific detection of 

*Trichophyton rubrum*.

Rapid Diagnosis of Dermatophyte Nail Infections.

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Key words

Dermatophyte, diagnosis, DNA extraction, onychomycosis, PCR, tinea unguium,

Trichophyton rubrum

Abstract

A rapid two step DNA extraction method and a multiplex PCR for the detection of dermatophytes in general and Trichophyton (T.) rubrum specifically were developed and evaluated on DNA extracted from pure cultures and from clinically diseased nails. DNA from the following dermatophytes were used: Epidermophyton floccosum, Microsporum (M.) audouinii, M. canis, M. gypseum, M. nanum, T. mentagrophytes, T. rubrum, T. schoenleinii, T. soudanense, T. terrestre, T. tonsurans, T. verrucosum and T. violaceum. Human DNA and DNA from the following non-dermatophyte fungi were included as controls: Alternaria, Aspergillus niger, Candida (C.) albicans, C. glabrata, C. krusei, Malassezia furfur, Saccharomyces cerevisiae, and Scopulariopsis brevicaulis. A total of 118 nail samples received for routine microscopy and culture for dermatophytes were subsequently tested by the two PCRs separately and in a multiplex format. Using DNA extracted from pure cultures the pan-dermatophyte PCR, the T. rubrum specific PCR sequentially and in a multiplex format correctly detected all dermatophytes and additionally correctly identified T. rubrum. Comparison of the traditional diagnostic evaluation (microscopy and culture) of nail samples with PCR on DNA directly extracted from the nails showed excellent agreement between PCR and microscopy, but the number of samples with dermatophyte species identification was increased considerably from 22.9% to 41.5%, mainly due to the identification of T. rubrum by PCR in
microscopy positive but culture negative samples. In conclusion, this 5-hour diagnostic
test was shown to increase not only speed but also sensitivity of investigation for nail
dermatophytosis.


Introduction

Human pathogenic dermatophytes are keratinophilic moulds that infect human skin, nails, and hair. Three genera exist: *Trichophyton*, *Microsporum*, and *Epidermophyton*, however, their predilection site for infections varies. Nail infections are mainly caused by *T. rubrum* followed by *T. mentagrophytes* (1,19,20,25,27,29) in contrast to hair and skin infections that may be caused by other dermatophyte species, including *Microsporum* spp. Prevalence ranges of onychomycosis in European countries vary between 3-22% (8,9,27). In addition to dermatophytes also *Candida* and non-dermatophyte moulds may be recovered from clinically affected nails. In a study by Summerbell et al. in which 2662 affected nails were examined, the following agents were isolated: *T. rubrum* (> 70%), *T. mentagrophytes* (20%), *Candida albicans* (5,5%), and *Scopulariopsis brevicaulis* or non-dermatophyte moulds (1.6%) (26). While the recovery of an anthropophilic dermatophyte should always be regarded as representing a true pathogen, *Candida* and non-dermatophyte moulds may represent contaminants, colonising agents or a secondary infection due to local or systemic factors (2,3,20). As other conditions, as for instance psoriasis, may resemble onychomycosis, and as onychomycosis, requires long term systemic antifungal treatment, the correct identification of casual fungi is mandatory (6,28). The current diagnosis is based on detection of fungal elements by direct microscopy of the clinical specimens followed by *in vitro* culture and morphological identification of the fungus (2,16,18). Direct microscopic examination of skin and nail material is often sufficient for the diagnosis of a fungal infection, but does not provide genus or species identification and hence does not differentiate unquestionably between dermatophytes and other moulds. Furthermore, although rapid and economical, this
technique is false negative in 5-15% of the cases (5, 21, 22). The subsequent species identification is performed by culture and morphological examination of colonies. The culture is, however, negative in up to 40% of the microscopy positive cases and is time consuming due to the slow growth and sporulation and the need for additional physiological tests (21, 28). Therefore, the time required for species identification may vary from 10-15 days up to 3-4 weeks (16).

A simple, rapid and specific method for the diagnosis of dermatophyte infections would obviously be a major improvement. Introduction of a PCR based methodology would increase specificity, simplicity, speed and potentially even reduce cost. For studies on species identification and typing, PCR (7, 11), PCR fingerprinting (4, 12), random amplification of polymorphic DNA (RAPD) (14, 17), PCR and restriction fragment length polymorphism (RFLP) (24, 12), arbitrarily primed PCR (AP-PCR) (16, 17) have all been applied. The main targets have been the following genes or DNA fragments: rDNA region, DNA topoisomerase II genes, and the chitin synthase gene (11, 13). Recently, Kardjeva and colleagues presented a 48-h diagnostic method of onychomycosis involving a 14-step nail pre-treatment and DNA extraction method and a subsequent T. rubrum specific PCR combined with RFLP and sequencing of the Internal Transcribed spacer (ITS) region for the detection of other fungal agents (15). Such a methodology is, however, difficult to implement in a routine laboratory receiving large numbers of nail specimens.

In this paper we present an alternative multiplex PCR based method especially developed for the detection of dermatophyte nail infections. In a 2-step extraction procedure followed by a single multiplex PCR and electrophoresis the method enables the diagnosis
of infection caused by any one of the dermatophytes (pan-dermatophyte) and in case of *T. rubrum* infection even a genus and species identification. An elaborated 2 step 15-minute DNA extraction method directly from patient samples allows application of this method in routine diagnostic laboratories.

**Materials and methods**

**Strains and clinical isolates.** Twelve fungal strains were purchased from the National Collection of Pathogenic Fungi (United Kingdom). Clinical isolates were obtained from Mycology Laboratory of Statens Serum Institute (SSI, Denmark) (Table 1). All clinical isolates were identified by observation of macro- and micro- morphology.

**Clinical nail samples.**

One hundred eighteen nail samples received for routine examination at the Laboratory of Mycology at SSI were prospectively included. The only inclusion criterion was the presence of sufficient amount of material for investigation by direct microscopy and culture as well as PCR analysis.

**DNA preparation from dermatophyte cultures.** The strains and clinical isolates were cultured in 2 ml of Sabouraud liquid medium with cyclohexamide and chloramphenicol (SSI Diagnostika, Denmark) and incubated with shaking for up to 8 days at 27°C. After harvest, pellet was re-suspended in 500 µL of lysis buffer (400 mM Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl, 1% sodium dodecyl sulphate) and left at room temperature for 10 minutes. 150 µL of potassium acetate (pH 4.8) was added and tubes
were vortexed and centrifuged (1 min, 12000 x g). The supernatant was transferred to a new tube and an equal volume of isopropyl alcohol was added. DNA pellet was washed in 70% ethanol. Dried DNA pellet was dissolved in 50 µL of TE (10 mM Tris, 1 mM EDTA) buffer. 2 µL of the DNA was used in 20-50 µL of PCR mixture. Reagents were, unless otherwise stated in the text, purchased from Sigma (Germany).

DNA preparation from nail samples (Copenhagen Patent office, patent pending no pct/DK2006/000332). DNA from nail samples was extracted by a 10 minute incubation of the nail sample in 100 µL of extraction buffer (60 mM sodium bicarbonate (NaHCO₃), 250 mM potassium chloride (KCl) and 50 mM tris (hydroxymethyl) amino methane (Tris), (pH 9.5) in 95°C and a subsequent addition of 100 µL anti-inhibition buffer (2% BSA). After vortex-mixing this DNA containing solution was used for PCR.

Pan-dermatophyte PCR (Copenhagen Patent office, patent pending no pct/DK2006/000332). Based on the comparison (VectorNTI, InforMax, Inc.) of nucleotide sequences of different dermatophytes presented in NCBI Nucleotide Database a set of primers detecting DNA fragment encoding Chitin Synthase 1, panDerm1 (5’GAAGAAGATTGTCGTTTGCATCGTCTC 3’) and panDerm2 (5’CTCGAGGTCAAAGCAGCAGCCAGAG 3’) was designed. 12 dermatophyte reference strains, 89 clinical dermatophyte isolates, 22 non-dermatophyte fungal isolates and purified human DNA (Table 1) were tested. PCR mixtures consisted of 10 µL of PCR Ready Mix (Sigma, Germany), 0.2 µL of each primer at concentration 100 µM (panDerm1 and panDerm2) and 4 µL of DNA in a volume of 20 µL. PCR was performed...
in a MWG-Biotech thermal cycler. The time-temperature profile of PCR was 45 cycles of
30 s at 94°C, 30 s at 60°C, 30 s at 72°C, preceding by initial denaturation 10 min at 95°C.
Presence of specific PCR products of approximately 366 bp was examined using
electrophoresis on 1% agarose gel and staining with ethidium bromide.

**Trichophyton rubrum specific PCR.** On the basis of alignment (VectorNTI (InforMax, Inc.) sequences of internal transcribed spacer 2 presented in NCBI Nucleotide Database, universal, uni (5’ TCTTTGAACGCACATTGCGCC 3’) and *Trichophyton rubrum* – specific Trubrum-rev (5’ CGGTCTCTGAGGGCGCTGAA 3’) primers were designed. Each reaction was performed in a volume of 20 µL by the addition of 4 µL of the DNA from microorganisms listed above, 0.2 µL of each primer (at concentration 100 µM) and 10 µL of PCR Ready Mix (Sigma, Germany). The amplification was performed in a
thermal cycler (MWG-Biotech, Germany) and consisted of one initial cycle of
denaturation for 5 min at 94°C and 45 cycles of 30 s at 94°C, 30 s at 60°C and 30 s of
extension at 72°C. After the thermal cycles, the amplicons were electrophoresed in a 2%
agarose gel and stained with ethidium bromide. To standardize the procedure, different
DNA concentrations and thermal cycles were tested (data not shown).

**Multiplex PCR.** The multiplex PCR was performed using the two specific sets of
primers described above: panDerm1 and panDerm2 primers, and uni and Trubrum-rev
primers. The reaction was performed at different conditions. 0.2 mM of each primer were
used. The following time-thermal profile was chosen: one initial cycle of denaturation for
5 min at 94°C and 45 cycles of 30 s at 94°C, 30 s at 60°C and 30 s of extension at 72°C.
After the thermal cycles, the amplicons were electrophoresed in a 2% agarose gel and stained with ethidium bromide. Specificity of multiplex PCR was tested on DNA from all of the strains listed in Table 1 and on human DNA. To standardize the procedure, different DNA concentrations and thermal cycles were tested (data not shown). The multiplex PCR (and separately the pan-dermatophyte and the *T. rubrum* specific PCRs) were subsequently evaluated using 97 nail specimens received for routine analysis.

**Results**

**Evaluation of pan-Dermatophyte and *T. rubrum* PCR using DNA extracted from fungal cultures.** Extracted DNA from cultures of 12 reference dermatophyte strains, 89 clinical dermatophyte isolates and 21 other fungi (Table 1) were used for evaluation of the pan-Dermatophyte primers and the *T. rubrum* specific primers separately as well as in a multiplex PCR format. A 203 bp PCR product corresponding to *T. rubrum* was observed for 13/13 *T. rubrum* DNA samples using the *T. rubrum* specific PCR separately and in multiplex format, and specific 366 bp PCR products were obtained for 101/101 dermatophyte DNA samples by the pan-dermatophyte PCR alone and in multiplex format (exemplary results of pan-Dermatophyte and *T. rubrum* specific PCRs are presented on Fig. 1). No PCR products were detected by the pan-dermatophyte PCR, the *T. rubrum* specific PCR nor the multiplex PCR for the 21 non-dermatophyte fungal isolates or from 3 samples of human DNA (100% sensitivity and 100% specificity for all three PCR systems).
Clinical evaluation of pan-Dermatophyte and *T. rubrum* PCR using DNA extracted directly from nail samples. By conventional diagnostics of the 118 nail samples 25 yielded growth of *T. rubrum*, 1 of *T. rubrum* and *T. mentagrophytes*, 1 of *T. tonsurans*, 3 of *Alternaria* sp., 1 of *Acremonium* sp., 1 of *Aspergillus* sp., 1 of *Candida* sp., 2 of *S. brevicaulis*, and 1 of a yeast species not further speciated. Sixty four were microscopy and culture negative and 18 were positive in direct microscopy for hyphae and conidia, but culture negative, why the latter samples were regarded as fungus positive (but no genus nor species identification could be established). Samples which were culture positive for non-dermatophyte fungi were regarded as dermatophyte negative by conventional methodology in the comparison with PCR results. DNA from 118 clinical samples was extracted using the elaborated rapid two-step protocol. For each of the samples again three set of PCRs were performed (*T. rubrum* specific PCR, pan-Dermatophyte PCR and multiplex pan-Dermatophyte+*T. rubrum* PCR). All multiplex PCR results were in agreement with the single PCR results indicating no loss of sensitivity in the multiplex PCR set-up. Overall, 50/118 (42.4%) of the samples were dermatophyte positive by PCR and 45/118 (38.1%) positive by traditional diagnostics including samples positive by microscopy but negative by culture. Among 24 specimens which were microscopy and culture positive (*T. rubrum*), 21 (87.5%) were confirmed by PCR as *T. rubrum* positive, two were PCR negative (8.3%) and one reported as *T. mentagrophytes* and *T. rubrum* positive by conventional examination was pan-Dermatophyte positive but *T. rubrum* PCR negative. Of 64 specimens negative by conventional microscopy and culture, 49 (76.6%) were confirmed by PCR as negative but 15 (23.4%) were PCR positive (*T. rubrum*). Within 18
microscopy positive but culture negative specimens (the presence of hyphae was observed), ten were *T. rubrum* PCR positive (55.6%), seven were negative by PCR (38.9%), and the result for one sample was not possible to interpret (5.6%) (unspecific PCR products were synthesised). Two specimens which were negative by microscopic examination of the nail but *T. rubrum* positive in culture were negative by PCR (exemplary results of pan-Dermatophyte and *T. rubrum* specific PCRs are presented on Fig. 2). One specimen diagnosed by conventional examination as *T. tonsurans* was pan-dermatophyte and *T. rubrum* PCR positive. Finally, PCR results for nine specimens diagnosed by conventional examination as non-dermatophyte species are presented in Table 2.

To investigate if the lack of PCR products in PCR negative samples could be due to the presence of PCR inhibitory substances in the samples, all PCR-negative specimens were spiked with *T. rubrum* DNA and subsequently re-tested in the multiplex pan-Dermatophyte-*T. rubrum* PCR. A PCR product was produced in all cases (data not shown). All the *T. rubrum* specific PCR products obtained from specimens not diagnosed as *T. rubrum* positive samples by traditional methodology were sequenced (MWG Biotech, Germany) and sequences of all of them matched that of *T. rubrum* reference strain (NCPF 113). The pan-Dermatophyte PCR product obtained from the DNA of the specimen diagnosed conventionally as *T. rubrum* and *T. mentagrophytes* was sequenced and the sequence matched that of *T. mentagrophytes* reference strain (NCPF 224). A comparison of results obtained by conventional diagnostics and PCR is shown in Table 3. Overall, the number of positive samples was increased by 11% (45 (38.1%) versus 50 (42.2%)) of 118 specimens were positive by conventional and PCR methodology,
respectively). Further more due to a considerable number of microscopy positive but
culture negative samples the percentage of samples with a species identification was
almost doubled by use of the PCR (49 out of 118 specimens were found *T. rubrum*
positive applying the PCR based method, while only 27 out of 118 specimens were
dermatophyte positive by culture).

**Discussion.**

A day to day detection of dermatophyte infection in nails is obviously a major
improvement in the diagnosis of tinea unguium allowing antifungal treatment to be
instituted promptly upon correct diagnosis and at the same time restricted to those with
dermatophyte infections. Although the species distribution varies between different
parts of the world *T. rubrum* is in most surveys reported to be the major pathogen in
tinea unguium accounting for 63-89% of the infections (1,9,10,19,23,27). On the same
time dermatophytes belonging to the less terbinafine susceptible genus of
*Microsporum* are unanimously reported to be very rare agents of onychomycosis and
the detection of dermatophyte DNA in a nail specimen will therefore represent
infection with a terbinafine susceptible dermatophyte in the vast majority of cases and
thus provide sufficient information guiding the clinician even despite lack of species
identification.

The clinical evaluation of single and multiplex PCR detection of any dermatophyte
and of *T. rubrum* specifically in nail specimens showed increased sensitivity when
compared to conventional diagnosis (Table 3). In this comparison samples positive by
microscopy but negative by culture were regarded as dermatophyte positive by
traditional diagnostics though we can not rule out that some of these cases may
represent non-dermatophyte infections. It is however not uncommon to obtain
negative culture results from patients with dermatophytosis, due to difficulties
associated with sampling (insufficient material, use of nail clippings instead of
subungual material), to prior medical treatment etc. and cases with positive
microscopy but negative culture should therefore always be investigated further (5).

On one occasion the species identification obtained by conventional culture and PCR
were conflicting (T. tonsurans by culture and T. rubrum by PCR). The fact that the T.
rubrum PCR was negative when applied on the T. tonsurans reference strain as well as
on all the clinical control T. tonsurans isolates tested initially raises the question if this
was a case of mis-identification by conventional identification or a double infection
with T. tonsurans and T. rubrum. As nail infections in Denmark caused by T.
tonsurans are extremely rare especially among Danes (as in this case) the former
explanation is the more likely in our opinion, however, the isolate was not stored and
thus no further examinations were possible. One specimen was by conventional
methodology diagnosed as a mixed infection with T. rubrum and T. mentagrophytes,
but the PCR yielded solely a pan-Dermatophyte PCR product. This was sequenced and
matched that of T. mentagrophytes in agreement with the culture result. Several
explanations for this apparent lack of detection of the T. rubrum isolate in this case
exist. 1) Although the specimens used for conventional and PCR testing derived from
the same patient is not exactly the same material and the T. rubrum may not have been
present in the specimen used for PCR. 2) This may be a case of contamination of the
culture plates by T. rubrum. 3) The sensitivity of the T. rubrum PCR may be
insufficient in cases of mixed infections. The facts, however, that the *T. rubrum*

specific primers target a multicopy gene in contrast to the pan-Dermatophyte primers

and that the *T. rubrum* PCR was also negative when the sample was run in a single

PCR set-up suggest that the sensitivity of the *T. rubrum* PCR should not be inferior to

that of the pan-Dermatophyte PCR. But examination of additional samples from cases

of documented mixed infections is necessary to evaluate this further.

The interpretation of the detection of non-dermatophyte moulds in nail specimens is

controversial. Such findings may reflect the presence of mould elements in the nail

specimen due to contamination, transient colonisation or infection of a traumatised or

otherwise diseased nail or it may reflect contamination in the laboratory. Therefore, at

least repeated recovery of the identical mould species is typically required before a

pathogenic role is considered and even in these cases the recovery may represent an

infection which is secondary to an underlying pathological nail condition. The finding

in this study that 2 nails yielded moulds by culture but *T. rubrum* by PCR may reflect

overgrowth by the rapid growing contaminating or colonising mould or true double

infection.

Although dermatophyte and/or *T. rubrum* identification in the nail specimens has been

attempted using a range of molecular methods, only one recently published study

involved DNA extraction directly from nail specimens without prior culture (15). The

extraction method described, however, was a multi-step procedure involving 14 steps and

thus labour intensive and per se associated with an increased risk of contamination. The

application of a 2-step 15 minutes DNA extraction procedure directly from nail

specimens and a multiplex PCR-based diagnosis of any dermatophyte and/or *T. rubrum*
with increased sensitivity compared to conventional diagnostic procedures allow for the first time integration of molecular biology-based method into the routine examination of nail dermatophytosis also for diagnostic laboratories receiving specimens on a larger scale. This brings hope that rapid, specific, and low cost diagnosis of onychomycosis may become broadly available in the near future.


amplification of polymorphic DNA (RAPD) and southern hybridization analyses. Mycoses 41:229-233.


Figure 1. Example of *Trichophyton rubrum* specific (lane 2-3) and pan-dermatophyte PCR products (lanes 4-11) analysis. Lanes: 1 and 12 - molecular weight marker (fragment sizes (bp): 501, 489, 404, 331, 242, 190, 147, 111, 110), 2-3 – results of *T. rubrum* specific PCR performed for *T. mentagrophytes* DNA (lane 2) and *T. rubrum* DNA (lane 3), lanes 4-11 – results of pan-dermatophyte PCR performed for: *M. audouinii* (lane 4), *T. mentagrophytes var. mentagrophytes* (lane 5), *T. schoenleninii* (lane 6), *T. terrestre* (lane 7), *T. rubrum* (lane 8), *T. tonsurans* (lane 9), *T. soudanense* (lane 10), *Epidermophyton floccosum* (lane 11).

Figure 2. Example of *Trichophyton rubrum* specific and/or pan-dermatophyte multiplex PCR products analysis. Lanes: 1 - molecular weight marker (100 bp DNA ladder), 2 – 6 – results of multiplex PCR performed for DNA extracted directly from nail specimens diagnosed by conventional methods as 2 – negative, 3 – *M. audouinii*, 4 – *T. rubrum*, 5 – *T. mentagrophytes*, 6 – *Aspergillus sp.*
Table 1. Microorganisms used in the study.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Number of reference strains (NCPF number)</th>
<th>Number of clinical isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>1 (NCPF-777)</td>
<td>14</td>
</tr>
<tr>
<td><em>M. audouinii</em></td>
<td>1 (NCPF-436)</td>
<td>5</td>
</tr>
<tr>
<td><em>M. canis</em></td>
<td>1 (NCPF-177)</td>
<td>10</td>
</tr>
<tr>
<td><em>M. gypseum</em></td>
<td>1 (NCPF-40)</td>
<td>2</td>
</tr>
<tr>
<td><em>M. nanum</em></td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>T. mentagrophytes var. interdigitale</em></td>
<td>1 (NCPF-780)</td>
<td>10</td>
</tr>
<tr>
<td><em>T. mentagrophytes var. mentagrophytes</em></td>
<td>1 (NCPF-224)</td>
<td>12</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>1 (NCPF-113)</td>
<td>12</td>
</tr>
<tr>
<td><em>T. schoenleinii</em></td>
<td>1 (NCPF-124)</td>
<td>-</td>
</tr>
<tr>
<td><em>T. soudanense</em></td>
<td>1 (NCPF-800)</td>
<td>13</td>
</tr>
<tr>
<td><em>T. terrestre</em></td>
<td>1 (NCPF-602)</td>
<td>8</td>
</tr>
<tr>
<td><em>T. tonsurans</em></td>
<td>1 (NCPF-690)</td>
<td>8</td>
</tr>
<tr>
<td><em>T. verrucosum</em></td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td><em>T. violaceum</em></td>
<td>1 (NCPF-794)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Other fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alternaria sp.</em></td>
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<td>1</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Malassezia furfur</em></td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Scopulariopsis brevicaulis</em></td>
<td>-</td>
<td>1</td>
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</tbody>
</table>
Table 2. PCR results for the nine clinical specimens with growth of a non-dermatophyte fungus.

<table>
<thead>
<tr>
<th>Results of routine examination</th>
<th>Culture</th>
<th>Number of isolates</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td><em>Acremonium</em></td>
<td>1</td>
<td>Neg</td>
</tr>
<tr>
<td>Pos</td>
<td><em>Alternaria</em></td>
<td>1</td>
<td>Neg</td>
</tr>
<tr>
<td>Neg</td>
<td><em>Alternaria</em></td>
<td>1</td>
<td><em>T. rubrum</em></td>
</tr>
<tr>
<td>Pos</td>
<td><em>Aspergillus</em></td>
<td>2</td>
<td>Neg</td>
</tr>
<tr>
<td>Pos</td>
<td><em>Candida sp.</em></td>
<td>1</td>
<td>Neg</td>
</tr>
<tr>
<td>Pos</td>
<td><em>S. brevicaulis</em></td>
<td>1</td>
<td>Neg</td>
</tr>
<tr>
<td>Pos</td>
<td><em>S. brevicaulis</em></td>
<td>1</td>
<td><em>T. rubrum</em></td>
</tr>
<tr>
<td>Pos</td>
<td>yeasts</td>
<td>1</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Pos: positive result
Neg: negative result

Table 3. Comparison of the results of conventional and PCR based examination of nail specimens.

<table>
<thead>
<tr>
<th>Result</th>
<th>Dermatophyte</th>
<th>Species specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td></td>
<td></td>
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<tr>
<td>Conventional</td>
<td>38.1% (45/118)</td>
<td>22.9% (27/118)</td>
</tr>
<tr>
<td>PCR based</td>
<td>42.4% (50/118)</td>
<td>41.5% (49/118)</td>
</tr>
<tr>
<td>Increase of detection</td>
<td>4.3%</td>
<td>18.6%</td>
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</tbody>
</table>