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MICROBIOLOGY

# Clinical application of a molecular assay for the detection of dermatophytosis and a novel non-invasive sampling technique

Alice Kizny Gordon[1], Christopher McIver[1,2], Minhee Kim[2,3], Dedee F. Murrell[2,3] and Peter Taylor[1,2]

[1]*Department of Microbiology, South Eastern Area Laboratory Services, St George Hospital, Sydney,* [2]*Faculty of Medicine, University of New South Wales, Sydney, and* [3]*Department of Dermatology, St George Hospital, Sydney, NSW, Australia*

**Summary**

The dermatophytoses are the most common superficial fungal infections worldwide. Clinical diagnosis is not reliable as there are many differentials, and laboratory diagnosis is required to gain access to treatment in more severe disease. Traditional diagnostic methods are limited by suboptimal sensitivity, specificity and prolonged turnaround times. Molecular methods are being used increasingly in the diagnostic algorithm in the clinical microbiology laboratory. The aim of this study was to evaluate a real-time polymerase chain reaction (RT-PCR) targeting the chitin synthase 1 gene (*CHS1*) of dermatophytes for analytical specificity, and to assess its clinical application by comparing it to the current methods of microscopy and culture. We also assessed a novel non-invasive sample collection technique involving adhesive tape impressions of suspected lesions. The PCR was highly specific, being able to discern between cultures of dermatophytes and other microorganisms. It also proved to be more sensitive than traditional methods at detecting dermatophytes in clinical samples. Similar sensitivities were seen on the samples assessed by the adhesive tape technique. An internal control system allowed for the detection of inhibition in certain culture and clinical specimens. This rapid and cost-effective technique could be incorporated into the initial diagnostic algorithm for dermatophytosis in Australian laboratories.

*Key words:* Adhesive tape; chitin synthase; dermatophytes; real-time polymerase chain reaction; tinea.

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## INTRODUCTION

Dermatophytes are a group of filamentous fungi that can infect skin, hair and nails. The group comprises around 20 species from three closely related genera – *Epidermophyton, Microsporum* and *Trichophyton*. These organisms cause infections including tinea capitis (infection of the scalp), tinea pedis (infection of the feet), tinea corporis (infection of the body), tinea cruris (infection of the groin area), and tinea unguium or onychomycosis (infection of the nails). It has been found that around 20–25% of the world's population are affected by superficial mycoses, of which dermatophytes are the most common.[1] Locally, an Australian study found the prevalence of onychomycosis in nursing home patients to be 22.5%.[2] In our laboratory, which provides service to two hospitals in metropolitan Sydney, Australia, we receive around 300 samples of hair, skin and nails per year for investigation of mycoses. With an ageing population and increasing use of immunosuppressive drugs, the dermatophytoses are emerging as an important cause of morbidity.

In Australia, a laboratory diagnosis confirming dermatophyte infection is required for access to subsidised systemic treatment for tinea capitis, onychomycosis or recalcitrant disease. Traditional diagnostic methods for dermatophytes include microscopy and culture, however both of these methods have their limitations. Microscopy is complicated by high numbers of false positive results, as it is unable to differentiate between dermatophytic and non-dermatophytic infections.[3,4] Isolation by culture, conventionally considered the gold standard, is limited by poor sensitivity and delayed results, taking up to 6 weeks for adequate growth. Histological diagnosis with periodic acid-Schiff (PAS) staining can also be performed, however this method can take more than 48 hours to prepare and it is not specific for the dermatophytes. Molecular diagnostics are becoming more widely accessible and have the advantage of superior sensitivity, specificity, and shorter turnaround times than conventional methods. Molecular techniques for dermatophyte diagnosis are being developed and used increasingly in the clinical microbiological laboratory setting.[5–13] These molecular techniques include conventional polymerase chain reaction (PCR) including post-PCR techniques such as electrophoresis or hybridisation probes to detect amplicons and real-time PCR (RT-PCR).[14] In Australia, Liu *et al.* [15–17] developed several arbitrarily primed PCRs to detect pan-dermatophytes, *Trichophyton* species and *Microsporum canis* in the research setting. To our knowledge there are no studies assessing the applicability of these tests in the clinical microbiological laboratory in the Australian setting.

In this study, a RT-PCR targeting a sequence of the chitin synthase 1 gene (*CHS1*), which is a highly conserved region across all dermatophytes,[18] was developed and assessed for analytical specificity. The clinical applicability was evaluated using clinical samples from suspected dermatophytoses

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collected over a 12-month period from St George and Sutherland Hospitals in Sydney, Australia. All samples were examined by traditional microscopy (including a subset stained by PAS) and culture, as well as PCR. A group of these patients were sampled for the PCR assay using a novel non-invasive technique involving a transparent cellulose-based pressure sensitive tape (adhesive tape).

## MATERIALS AND METHODS

### Ethics

Ethics approval for this study was granted by the Human Ethics Research Committee of South Eastern Sydney Local Health District (St George Hospital), Sydney, Australia.

### Source of microorganisms for validation

A genetically diverse range of microorganisms was used to validate the PCR assay for the detection of dermatophyte DNA (Table 1). Culture isolates of a range of filamentous fungi commonly associated with dermatophyte infections ($n = 50$) and those of no clinical significance ($n = 16$) were tested. These strains were obtained from subcultures of aqueous suspensions of previous clinical isolates; culture isolates from two collaborating hospital laboratories and The National Reference Laboratory for Mycology, Adelaide. Other microorganisms included strains of yeast ($n = 11$) and bacteria ($n = 36$), which were sourced from a large laboratory collection of clinical microorganisms stored in suspensions of 10% nutrient broth glycerol at −70°C.

**Table 1** Microorganisms tested to assess specificity of PCR assay for dermatophytes and corresponding results

| Microorganism | *n* | PCR positive[a] |
|---|---|---|
| Filamentous fungi | | |
| Dermatophytes | 50 | 47/50 |
| *Epidermophyton flocosum* | 8 | 8/8 |
| *Microsporum canis* | 3 | 0/3 |
| *Microsporum gypseum/fulvum* complex | 2 | 2/2 |
| *Trichophyton interdigitale* | 6 | 6/6 |
| *Trichophyton mentagrophytes* group | 8 | 8/8 |
| *Trichophyton rubrum* | 9 | 9/9 |
| *Trichophyton tonsurans* | 10 | 10/10 |
| *Trichophyton violaceum* | 4 | 4/4 |
| Non-dermatophytes | 16 | 0/16 |
| *Alternaria* sp. | 2 | 0/2 |
| *Cladosporium* sp. | 1 | 0/1 |
| *Curvularia* sp. | 2 | 0/2 |
| *Exophiala* sp. | 1 | 0/1 |
| *Exserohilum* sp. | 2 | 0/2 |
| *Fusarium* sp. | 1 | 0/1 |
| *Hortaea werneckii* | 1 | 0/1 |
| *Paecilomyces* sp. | 1 | 0/1 |
| *Penicillium* sp. | 1 | 0/1 |
| *Phaeoacremonium* sp. | 1 | 0/1 |
| *Phialophora* sp. | 2 | 0/2 |
| *Trichoderma* sp. | 1 | 0/1 |
| Yeast[b] | 11 | 0/11 |
| Bacteria[c] | 36 | 0/36 |

[a] Detection of the dermatophyte-specific chitin synthase 1 (*CSH1*) gene.
[b] *Candida albicans* ($n = 3$); *Candida guilliermondii* ($n = 1$); *Candida krusei* ($n = 1$); *Candida parapsilosis* ($n = 1$); *Cryptococcus gattii* ($n = 1$); *Cryptococcus neoformans* ($n = 2$); and *Saccharomyces cerevisiae* ($n = 2$).
[c] Gram positive cocci: *Enterococcus faecium* ($n = 3$); *Staphylococcus aureus* ($n = 7$); and *Staphylococcus* sp. (coagulase negative) ($n = 3$). Gram positive bacilli: *Listeria monocytogenes* ($n = 1$). Gram negative cocci: *Neisseria gonorrhoeae* ($n = 1$). Gram negative bacilli (*Enterobacteriaceae*): *Escherichia coli* ($n = 2$); *Citrobacter* sp. ($n = 1$); *Enterobacter* sp. ($n = 1$); *Klebsiella pneumoniae* ($n = 1$); *Plesiomonas shigelloides* ($n = 1$); *Proteus mirabilis* ($n = 1$); *Providencia stuartii* ($n = 1$); *Shigella boydii* ($n = 1$); and *Shigella flexnerii* ($n = 1$). Gram negative bacilli (miscellaneous): *Acinetobacter* sp. ($n = 2$); *Aeromonas sobria* ($n = 1$); *Eikenella corrodens* ($n = 1$); *Haemophilus influenzae* ($n = 1$); *Haemophilus paraphrophilus* ($n = 1$); *Legionella longbeachae* ($n = 1$); *Legionella pneumophila* ($n = 1$); *Pseudomonas aeruginosa* ($n = 1$); *Ralstonia pickettii* ($n = 1$); and *Roseomonas* sp. ($n = 1$).

### Mycological methods

*Sample collection*

Infected skin was sampled by drawing the sharp edge of a sterile scalpel blade across a lesion (skin scraping) and collecting in a 30 mL sterile specimen jar. For a group of unselected patients attending a dermatology clinic, an impression of the lesion was taken using a 1 $cm^2$ segment of commercial transparent cellulose-based pressure-sensitive tape (adhesive tape; Office Works, Australia) to which a paper tag was attached to facilitate handling (Fig. 1). The tag was removed before extraction (using sterile scissors). Infected nails were sampled using sterile nail clippers and where possible material beneath the nail was also collected.

*Microscopy*

Clinical samples were treated with 10% potassium hydroxide (KOH) for approximately 2 h to dissolve the keratin and to facilitate examination for fungal elements using phase microscopy. A subset of nail specimens was fixed in paraffin, sectioned, stained by the PAS reaction and examined using conventional microscopy.[19]

*Culture*

Sabouraud's dextrose agar (Oxoid, UK) supplemented with gentamicin (0.05 g/L) alone and in combination with chloramphenicol (0.05 g/L); and Mycosel agar (Becton Dickinson, USA) supplemented with cycloheximide (0.4 g/L) and chloramphenicol (0.05 g/L), were used to culture clinical specimens. Cultures were incubated for five weeks at 30°C, and examined weekly. Filamentous fungi were identified using criteria and methods described by Ellis *et al.*[20] and Larone.[21]

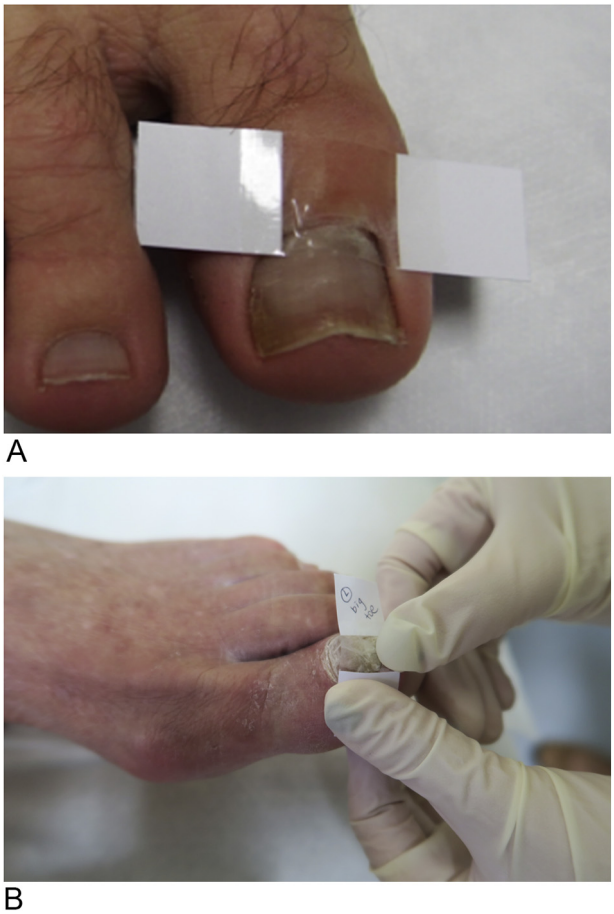

**Fig. 1** (A,B) Adhesive tape collection technique.