

## Effects of Modified Sample Collection Technique on Fungal Culture Yield: Nail Clipping/Scraping versus Microdrill

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### Introduction

Onychomycosis is the most frequent and treatable cause of nail disorders with a prevalence varying from 3%-20%.<sup>1,2</sup> Effective treatment requires long-term systemic therapy with antifungal agents at considerable cost to the patient with diverse adverse effects and the possibility of long-term treatment resistance. Moreover, with the advent of more effective systemic therapy, increasing numbers of clinicians are treating clinically suspected onychomycosis.<sup>3-7</sup> Only 50% of dystrophic nails have a fungal cause<sup>8,9</sup>, therefore it is imperative to establish a correct diagnosis by microscopy and culture before a patient is treated with a systemic antifungal agent. Although positive microscopy is often used as a criteria to initiate therapy, cultural identification of an offending organism is desirable and can have important treatment implications. Fungal culture, however, is frequently negative despite positive microscopy and a high index of clinical suspicion. The reasons for culture failure include the presence of non-viable fungi in the distal nail, inadequate or improper sampling and the presence of possible interfering substances or microorganisms.<sup>10,11</sup> Clinicians, therefore, should be aware of the need to collect an adequate amount of suitable clinical material.

Numerous methods of obtaining specimens for culture in cases of distal and lateral subungual onychomycosis have been described.<sup>11-16</sup> Most frequently used methods to obtain nail and subungual debris include nail clipping, scraping and subungual debris.<sup>2,13,17,18</sup> These collection methods, however, may not result in adequate yield in proximal onychomycosis, disto-lateral onychomycosis and in severely dystrophic nails. Moreover, fungi usually grow most actively at the proximal part of the affected nail<sup>17,19-21</sup>, which at times is difficult to sample particularly in disto-lateral onychomycosis and in dystrophic nails. To increase specimen yield, Epstein<sup>22</sup> suggested using a microdrill, while English and Atkinson<sup>20</sup> used a microdrill with a suction nozzle attached. Using these techniques, the Atkinson<sup>20</sup> and Epstein<sup>22</sup> showed that it is possible to obtain a specimen from a

definitive area of the nail with a resultant increased frequency of successful mycological culture despite a low rate of fungal hyphae detection by direct microscopy. Since then, many<sup>18,23,24</sup> have advocated the use of the microdrill technique to obtain specimens in all types of onychomycosis with special emphasis on proximal and dystrophic onychomycosis. To our knowledge, there have been only few comparative studies of the nail clipping and drilling methods.<sup>20,25,26</sup> The aim of this study was to investigate the utility of nail plate/subungual microdrilling as an alternative to conventional nail clipping/subungual scraping and to directly compare the frequency of KOH microscopy and fungal culture yielded by both methods.

### Materials and Methods

**Patient Selection** Forty six clinically suspected cases of onychomycosis from the clinics of dermatology at The Aga Khan University Hospital, Karachi Pakistan and outside referral patients from various hospitals of the city were prospectively evaluated over a 15 month period (from March 1998 to June 1999). Patients on topical antifungal therapy within the last 1 week or systemic antifungal therapy within the last 4 weeks were not included in the study. A detailed patient history, clinical examination, and relevant investigations were recorded on a pre-designed Performa at the screening visit. **Specimen Collection** The most severely affected nail was selected as the target nail for sampling for direct microscopy and culture by two different methods: clipping and drilling. When both toe and finger nails were affected, specimens were collected from both sites after selecting the target nails. The nail was first cleaned with 70% alcohol, after which the most distal part of the nail was removed and discarded. In the clipping technique, several small pieces of the affected nail were taken with the nail cutter and nail was scraped with a scalpel; in addition, debris from beneath the nail was scraped. In the drilling technique, a microdrill (Figure 1) without any suction device attached was used. The needle of the microdrill was autoclaved in between each patient. The nail was

drilled to a considerable depth at the growing edge as well as the center of the affected area to obtain a well represented specimen. In the same specimen, debris from beneath the nail was also carefully drilled. To obtain comparable nail specimens, these were taken from the same most affected nail, clipping first followed by drilling. Figure 2 demonstrates a post nail clipped and microdrilled patient's nail. Microscopic Examination and Culture Microscopic examination of the collected nail samples was performed using 20% potassium hydroxide (KOH) solution under the light microscope. Fungal culture was performed using three test media: 1) Sabouraud's dextrose agar medium with 0.5% chloramphenicol without cyclohexamide (at 25°C and 37°C); 2) 5% Sheep blood agar (at 37°C) and 3) Dermatophyte test medium (at 37°C). Both nail clippings and drilled powder were inoculated separately on to the media through a scalpel blade. The culture Petri dishes were incubated for 1-4 weeks. All moulds and dermatophytes were identified by gross colony morphology and microscopy examination with lactophenol cotton blue preparation. All yeasts were identified using Twin80 agar. Positive culture was defined as the presence of pure growth of either a dermatophyte/non-dermatophyte fungus or yeast at more than 5 out of 20 or 20% of the inoculum's site with or without corresponding positive microscopy. 25 Statistical Analysis The results of KOH microscopy and culture by nail clipping /scraping and microdrill were statistically analyzed using Fisher's Exact test (2 sided). A P value of <0.05 was considered statistically significant.

## Results

The demographic data and site of involvement of onychomycosis in all patients are summarized in Table 1. Female outnumbered males. Four of 46 (9%) patients were children between 3-6 years of age. House wives dominated over other patient's population. Finger nails were the most common site of involvement. None of the patients were HIV positive. One patient had poorly controlled type II diabetes mellitus and another patient had recently completed chemotherapy treatment for multiple myeloma. KOH Microscopy On microscopic examination (Table 2), 8 of 46 (17%) specimens were positive by combining the results of nail clipping and microdrill. Of the 8 KOH microscopy positive cases, 4 (50%) were positive by both method, 3 cases were positive by microdrill alone, while 1 case was positive by nail clipping/scraping alone. The difference in the number of positive KOH microscopy using nailclipping/scraping and microdrill methods was not statistically significant (P=0.379). Of the 7 specimens positive for microscopy by drilling, one case grew *Trichophyton rubrum*, 2 cases grew *Trichophyton*

Table1. Patients Demographics and

### sites of involvement of onychomycosis in all (46) patients.

Sex	Male	16
	Female	30
Age (Years)	Range	3-74
	Mean	36-4
	Occupation	
Occupation	House wives	13
	Students	7
	Miscellaneous	11
	None	5
Site	Finger nail	24 (52%)
	Toenail	18 (39%)
	Finger and toe	4 (8%)

Table2. Microscopy and fungal culture results by type of specimen collection(Nail clipping and Microdrilling).

Clipping (46)	Positive 5	2	1	2
	Negative 41	0	17	24
Microdrill (46)	Positive 7	3	1	3
	Negative 39	0	16	23
Clipping and Drilling (46) (results combined)	Positive 8	3	1	4
	Negative 38	0	18	20

mentagrophyte and one case grew *Candida parapsilosis*. Of the 5 cases positive for microscopy and clipping alone, 2 cases grew *Trichophyton mentagrophyte* and one case grew *Candida parapsilosis*. Nineteen of 38 (50%) microscopy negative specimens were culture positive. All microscopy negative but culture positive cases grew yeast organisms.

Mycological Culture Mycological cultures (Table 2) were positive in 48% cases by combining the results of microdrill and nail clipping (Table 1). Of the 22 positive cultures cases, 18 were positive by both methods while 2 were positive by the clipping technique alone and 2 were positive by the drilling technique alone. Hence, by combining the results of both techniques two additional cases were detected compared with clipping or drilling techniques alone. Organisms isolated are listed in Table 3. Yeasts were the predominant isolates (82.7%) and only 3 cases (13%) grew

Table3. Organisms isolated from fungal culture in onychomycosis in order of frequency.

Non-Dermatophytes	20	87
<i>Candida parapsilosis</i>	9	39.2
<i>Candida tropicalis</i>	6	26.1
<i>Candida albicans</i>	2	8.7
<i>Candida guilliermondii</i>	2	8.7
<i>Aspergillus terreus</i>	1	4.3
Dermatophytes	3	13
<i>Trichophyton mentagrophytes</i>	2	8.7
<i>Trichophyton rubrum</i>	1	4.3

dermatophytes, including 2 cases of *Trichophyton mentagrophyte* and 1 case of *Trichophyton rubrum*. Of the yeasts, *Candida parapsilosis* was the most common isolate (47%). Of the 22 positive cultures, 14 cases (64%) were obtained from finger nails (yeast n=13, mould n=1), 7 cases (32%) were obtained from toe nails (yeast n=5, dermatophyte n=2) and 1 case (4%) involved both the finger and toe nails (dermatophyte). Overall, the growth of yeast from the microdrill powder specimen was considerably

heavier, in the order of 8 to 10 times the quantity seen in conventional nail clipping/scraping culture, in over two thirds of the fungal culture plates (Figure 3). One case of *Candida parapsilosis* also grew *Aspergillus terreus*.

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## Abstract

### Objective:

Onychomycosis requires accurate diagnosis but fungal culture yield is frequently low by routine sampling techniques. The aim of this study was to investigate the utility of nail plate/subungual microdrilling as an alternative to conventional nail clipping/subungual scraping.

### Methods:

Patients with clinical evidence of onychomycosis (n=46) were prospectively evaluated for fungal potassium hydroxide (KOH) microscopy and culture comparing two sampling techniques: nail clipping versus microdrilling.

### Results:

Fungal cultures were positive in 48% with 2 additional cases detected by combining both methods. KOH microscopy was positive in 17% cases. Specimen obtained via the microdrill technique gave consistent heavier fungal growth on culture media. *Candida* species were the most common isolates (82.7% of cases) and were

negative on KOH microscopy in 95 % of culture proven cases. The microdrill technique yielded consistent heavier growth on culture media.

**Conclusion:**

Microdrill technique improves laboratory diagnosis and ultimately treatment of onychomycosis, particularly in patients with repeated KOH microscopy and culture failure despite strong clinical suspicion (JPMA 54:301;2004).