

# An In-Office Diagnostic Procedure To Detect Dermatophytes In a Nationwide Study of Onychomycosis Patients

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## ABSTRACT

**Purpose:** To evaluate in-office dermatophyte test medium (DTM) culture as an alternative to traditional laboratory fungal culture for confirming a diagnosis of onychomycosis, and to determine the prevalence of dermatophytes as a cause of onychomycosis in patients not participating in a clinical trial.

**Design:** This nationwide multicenter prospective study enrolled 1100 adult patients with suspect onychomycosis. DTM and laboratory fungal culture results were compared for individual patients.

**Methodology:** The 310 participating physicians obtained patient nail-bed specimens and divided them for testing by both diagnostic methods. The paired results of the two culture methods were compared using the kappa statistic.

**Principal findings:** Paired culture results were available for 975 of the 1100 enrolled patients. DTM results agreed with central laboratory cultures in 70 percent of cases. The kappa value of 0.40 indicated a moderate degree of correspondence between the two testing modalities. Overall, DTM culture indicated a dermatophyte in 616 patient specimens (56 percent) and central laboratory culture identified a dermatophyte in 528 of the specimens (48 percent). For the entire study population, dermatophytes were identified in 93 percent of the positive central laboratory cultures, confirming that dermatophytes caused the vast majority of the infections. The cost of each DTM culture was approximately \$1, compared to \$25 for each laboratory fungal culture.

**Conclusion:** This study demonstrates that the in-office DTM culture for diagnosing onychomycosis has comparable utility to the traditional laboratory fungal culture, is less expensive, and yields faster results.

**Key terms:** onychomycosis, dermatophytes, dermatophyte test medium, DTM, fungal infection, mycology.

## INTRODUCTION

Onychomycosis, a persistent fungal infection of the nail bed, is the most common nail disorder in adults, accounting for up to 50 percent of all nail diseases.<sup>1-3</sup> The causative agents of onychomycosis include dermatophytes (fungi that invade only dead tissues of the skin, nails, or hair), non-dermatophyte moulds, and rarely,

yeasts of the *Candida* species.<sup>4</sup> The dermatophytes *Trichophyton rubrum* and *T. mentagrophytes* are the most common causative pathogens of onychomycosis (also called tinea unguium), with *T. rubrum* responsible for approximately 90 percent of cases.<sup>1,2</sup>

Onychomycosis is likely to be encountered by the primary care practice, as it is frequently treated by the primary care physician. The overall prevalence of onychomycosis ranges from 2 percent to 14 percent, but it increases with age. Fifteen to twenty percent of persons between the ages of 40 and 60 have the condition, compared with 32 percent of persons between ages 60 and 70, and 48 percent of persons who are older than age 70.<sup>1</sup> Recent evidence suggests that the overall incidence of onychomycosis is increasing.<sup>1,2</sup>

Effective antifungal treatments are available, and these infections can usually be managed in the primary care setting with no need for referral. Several conditions can mimic onychomycosis, including psoriasis, nail trauma, contact irritants, and lichen planus. Consequently, an accurate, reliable, and inexpensive diagnostic test for dermatophytes would be extremely helpful. As noted by Daniel and Elewski in a recent editorial in the *Archives of Dermatology*, utilization of diagnostic tools for onychomycosis lags recent therapeutic advances.<sup>5</sup>

The traditional diagnostic test for onychomycosis is direct microscopy using potassium hydroxide (KOH) to visualize fungal hyphae and fungal culture.<sup>1,5,6</sup> While direct microscopy is quite sensitive, it is not useful in dis-

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tinguishing dermatophytes from other fungal organisms and necessitates some degree of interpretive skill to be properly performed. Additionally, direct microscopy is not suited for use in the office practice. Traditional fungal culture provides definitive identification of the pathogen, but the sensitivity of fungal cultures can be relatively low. Fungal cultures are comparatively expensive; require 3 to 6 weeks of growth for colonies to develop; and reproductive structures, conidia, must be present for species identification.

The DTM culture has been available since 1969.<sup>7</sup> DTM culture systems are commercially available that are suitable for use in the general-practice office, but are currently underutilized.<sup>8</sup> DTM culture does not identify specific organisms, but a positive DTM culture does confirm the presence of dermatophytes, which account for the vast majority of cases of onychomycosis. The advantages of DTM culture include ease of use and interpretation of results, faster turnaround time than other types of fungal culture, and low cost. The majority of DTM cultures can be identified after 1 week, and less than 2 percent of cultures require 2 weeks.<sup>7</sup> The DTM culture costs approximately \$1 per test (e.g., the ACU-DTM product used in this study), which is substantially lower than the charge from a central laboratory for mycologic cultures (\$25 per culture in this study). This is not a technically difficult diagnostic test; the operator does not need extensive training and can easily perform it in the office. DTM culture is highly specific if results are read within 14 days.<sup>7</sup>

We conducted this large, prospective nationwide study to evaluate in-office DTM culture compared to central laboratory fungal culture for confirming a clinical diagnosis of onychomycosis. To our knowledge, this is the largest prospective mycologic study conducted in community-practice patients presenting with

signs and symptoms of onychomycosis. This study population may be more representative of onychomycosis seen in the community than are study populations comprising patients treated in clinical trials with restrictive inclusion and exclusion criteria.

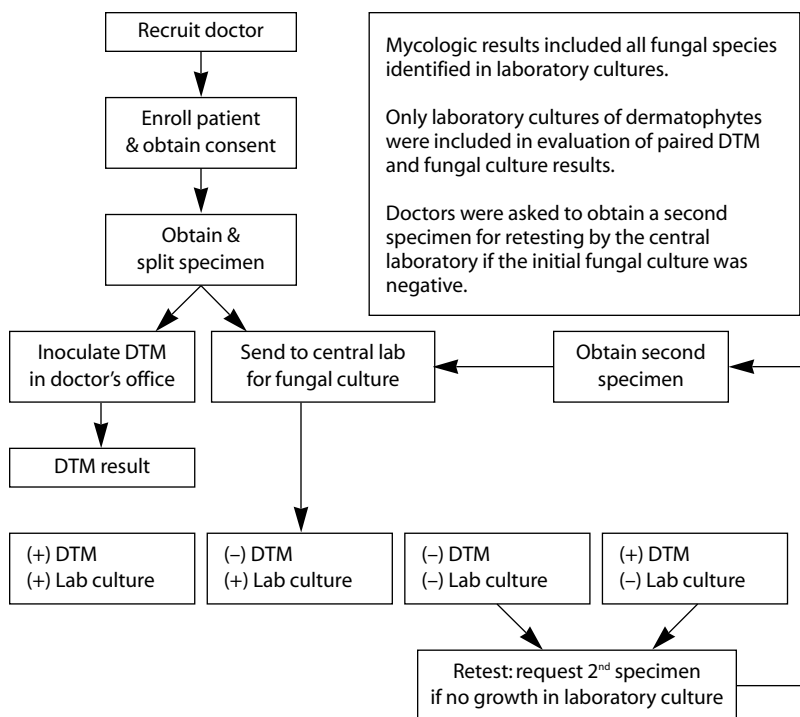
## METHODS

**Study design.** A total of 310 office- and clinic-based primary care physicians, dermatologists, and podiatric physicians participated in this study. The physician sample was stratified to ensure closely balanced geographic diversity. Each physician was asked to enroll five adult patients (18 years or older), and those who enrolled at least one patient are included in this study. Patients who had been treated with an oral antifungal agent within the last 90 days or a topical antifungal agent within the last 30 days were not eligible. Patient enrollment began on July 1, 2000, and data collection continued through Nov. 9, 2001.

At the initial office visit, the physician explained the nature of the study, obtained written informed consent, and collected demographic information and a relevant medical history. The clinician then obtained a specimen from the toenail bed for mycologic evaluation. Specimens were split, with half cultured on DTM in the office (ACU-DTM, Acuderm, Inc., Ft. Lauderdale, Fla.). The remaining specimen was sent to the Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, for Sabouraud glucose agar (SGA) culture and KOH evaluation. The overall study design, disposition of specimens, and testing procedures are shown in Figure 1. The Western Institutional Review Board, Olympia, Wash., approved the study protocol, an informed consent for the patient's review and signature, and all study materials.

Results of DTM culture were available in 2 weeks or less, and clinicians were informed of the culture results

**FIGURE 1** Infection confirmation study design and procedures



from the central laboratory 4 to 6 weeks after the sample was obtained. The initiation of antifungal therapy and the selection of a treatment were at the physician's discretion. If the central laboratory culture result was negative, physicians were asked to obtain a second sample for a repeat culture. The primary analyses conducted in this study were: a) a paired comparison of laboratory fungal culture and in-office DTM culture for each patient having results from both methods; and b) tabulation of the culture results and the infectious organisms that were identified.

**Mycologic evaluations.** Physicians were supplied with DTM culture kits and a videotape to instruct them on how to obtain the nail-bed sample and inoculate the DTM tube. The specimen was obtained after cleaning the surface of the nail plate with an alcohol swab and cutting the nail plate with a sterilized curette or clipper to expose the nail bed. To increase the likelihood of detecting any dermatophytes that might be present, doctors were instructed to obtain samples consisting of pieces of subungual debris from the proximal portion of the nail bed underneath the nail plate, using a probe or curette. The specimen was divided. One piece was sent to the central laboratory. The other piece was pressed lightly on the culture medium in a DTM tube, and the cap was replaced loosely to avoid sealing the tube off from the atmosphere. The specimen was incubated at room temperature for up to 2 weeks following the test-kit manufacturer's instructions. The physicians were advised to check the DTM culture daily for a change of color, which was interpreted as a positive result. False-positive results were avoided by examining the medium for the growth of white colonies, typical of dermatophytes, and by completing all readings by 14 days, after which time overgrowth by nondermatophyte organisms may occur. Patients with a positive KOH evaluation, negative

laboratory culture, and a DTM result, whether positive or negative, were asked to submit a second specimen for culturing.

The University of Texas Fungus Testing Laboratory performed its evaluations using well-established methods. Fungal culture was carried out using two media: one that contained cycloheximide, to inhibit nondermatophyte pathogens; and a cycloheximide-free, Sabouraud glucose agar, to allow the growth of yeasts and nondermatophyte fungi, which are other pathogens that can cause onychomycosis. Lack of growth of reproductive colonies within 4 to 6 weeks confirmed a negative fungal culture.

**Statistical analysis.** The primary objective of this study was to evaluate in-office DTM culture to confirm a clinical diagnosis of onychomycosis. For comparison of paired DTM and fungal culture results, positive DTM results were noted to agree with cultures that grew a dermatophyte organism, i.e., *T. rubrum*, *T. mentagrophytes*, or *Epidermophyton floccosum*. Negative DTM were noted to agree with growth of nondermatophytes or lack of growth in Sabouraud glucose agar culture. Cultures of nondermatophyte moulds were included in the epidemiologic results only. Agreement of the DTM and culture methods was estimated using the kappa statistic.<sup>9</sup> The asymptotic standard error and 95 percent confidence limits for kappa were calculated using the SAS software.<sup>10</sup> For binary data such as the DTM culture and central laboratory results reported here, kappa is a better measure of concordance than a correlation coefficient, which measures trends rather than concordance.<sup>9</sup>

## RESULTS

The 310 participating physicians each enrolled at least one patient in the study. One hundred forty-six practitioners (47 percent) were primary care physicians, 118 (38 per-

cent) were podiatric physicians, and the remaining 46 (15 percent) were dermatologists. A total of 1100 patients with signs and symptoms of onychomycosis were enrolled. The comparison of in-office DTM culture and fungal culture was based on the 975 patients (89 percent) for whom complete data consisting of paired DTM and laboratory culture results were available by the cutoff for data collection. All 1100 patients were included in the compilation of demographic and epidemiologic results.

Men and women were about equally represented. A majority of patients (82 percent) were Caucasian. Of note, 45 percent of the patients were age 65 or over, and 16 percent were diabetic. A median of 4.8 toenails per patient were affected, and 60 percent of patients had involvement of both feet. A similar average number of toes were affected on the right and left foot, 2.5 versus 2.4. Ten percent also had clinical evidence of fingernail onychomycosis, and 31 percent had symptoms of tinea pedis (Table 1). Fingernail involvement and the presence of tinea pedis were clinical observations only and were not confirmed by DTM or fungal culture.

Central laboratory culture results were positive in 48 percent (528 of the 1,100 patients). Three dermatophyte species, *T. rubrum*, *T. mentagrophytes*, and *E. floccosum*, accounted for 93 percent of the positive cultures (Table 2). Nondermatophyte moulds accounted for the remainder of isolates, and no *Candida* infections were identified. We observed no regional differences in the causative organisms of onychomycosis.

DTM and laboratory cultures were in agreement (i.e., both positive [ $n = 361$ ] or both negative [ $n = 321$ ]) in 70 percent of the 975 patients for whom paired results were available at the time of data analysis, for a kappa of 0.40 (asymptotic standard error = 0.037, 95 percent confidence interval: 0.305, 0.451 (Table 3). Overall,

the DTM cultures were positive in more cases than the laboratory cultures were — 56 percent (616/1100) versus 48 percent (528/1100).

Men had positive results by both culture methods more often than women (men, 64 percent for DTM and 59 percent for laboratory culture; women, 48 percent and 34 percent respectively). No other demographic variables were associated with the likelihood of a positive DTM or central laboratory culture result.

Laboratory cultures were negative in 572 patients. A second specimen was requested from these patients, and laboratory results were available for 137 patients at the time of data analysis. The retest culture was positive in 38 patients, 28 percent of the total who were retested. Of these, 18 of the positive retests were in patients who had a negative DTM culture, and 19 were in patients whose DTM culture was initially positive (Table 4). One retest culture grew a nondermatophyte mould. Retested patients were more likely to be female than male (58 percent versus 42 percent), but otherwise did not vary from the demographic profile of the entire population.

**DISCUSSION**

This large prospective multicenter (310-site) study demonstrates the utility of the DTM culture in confirming onychomycosis in a community-practice setting. It is the largest study of this type to establish the concordance of DTM culture with central laboratory fungal culture. Central laboratory culture and DTM were in agreement in 70 percent of the patients. The kappa coefficient of 0.40, a measure of agreement between multiple tests, indicates a moderate degree of agreement beyond that which would occur by chance. Overall, the DTM cultures were positive in 56 percent of patients, and central laboratory cultures were positive in 48 percent. The survey also confirmed dermatophytes as the primary

**TABLE 1 Patients enrolled in the infection confirmation survey: Demographic and clinical characteristics (n = 1100)**

Characteristic	N	% of patients	
Gender	male	561	51
	female	539	49
Race	white	859	78
	black	120	11
	hispanic	121	11
Age (y)	≥65	495	45
	55–64	220	20
	35–54	286	26
	18–34	99	9
Referring physician	primary care	146	47
	podiatrist	118	38
	dermatologist	46	15
Clinical characteristics	diabetes	165	16
	ingernail involvement	110	10
	Tinea pedis present	341	31

**TABLE 2 Pathogens identified by central laboratory culture in 528 patients with onychomycosis symptoms and positive cultures**

Organism	N	% of patients	
Dermatophytes	<i>T. rubrum</i>	448	85
	<i>T. mentagrophytes</i>	32	6
	<i>E. floccosum</i>	11	2
Nondermatophyte moulds	<i>Scopulariopsis brevicaulis</i>	11	2
	<i>Paecilomyces lilacinus</i>	5	1
	all others	21	4

**TABLE 3 Comparison of in-office DTM culture and central laboratory mycologic culture (n = 975)**

Percentages represent paired test results.\*

		Central laboratory culture		
		Positive	Negative	Total
DTM	Positive	37% (361/975)	19% (185/975)	56% (546/975)
	Negative	11% (107/975)	33% (321/975)	44% (429/975)
	Total	48% (468/975)	52% (507/975)	

\*A positive result is defined as dermatophyte growth in fungal culture or a color change in DTM culture. A negative result is defined as no growth or growth of a nondermatophyte in fungal culture, or lack of color change by 2 weeks in DTM culture.

pathogen in onychomycosis, accounting for 93 percent of infections, with nondermatophyte moulds accounting for the rest. No cases of

*Candida* onychomycosis were identified in the central laboratory cultures. The findings in this study support the adoption of the in-office DTM

**TABLE 4. Patients with completed DTM and central laboratory culture results who were retested (n=137 fungal culture results)**

DTM results	Central lab results	Retested central lab results
		negative = 72% (99/137)
negative	negative	negative = 38% (52)
positive	negative	negative = 34% (47)
		positive = 28% (38/137)*
negative	negative	positive = 13% (18) <sup>†</sup>
positive	negative	positive = 14% (19)

\* One of the 38 cultures was not a dermatophyte.

<sup>†</sup> Does not include the nondermatophyte.

for confirming a diagnosis of onychomycosis in patients presenting with clinical symptoms.

Many managed care organizations require a KOH test for reimbursement of therapy for onychomycosis. If the test is negative and onychomycosis is still suspect, a fungal culture may be ordered. The use of in-office DTM culture to confirm clinically suspect onychomycosis would provide an equivalent level of confirmation at a much lower cost, more quickly and more conveniently. In the present study, 653 patients (67 percent) with onychomycosis symptoms had positive results on either laboratory or DTM culture. If we assume these positive results were all accurate and patients had a prior probability of infection of 50 percent, we can calculate an upper bound for the sensitivity of each test. Based on these assumptions, fungal culture identified, at a maximum, 81 percent of true positives (528/653), while DTM culture identified 91 percent (616/653). In their description of the DTM culture method, Taplin et al<sup>7</sup> reported that 610 paired cultures, 211 fungal cultures (35 percent), and 240 DTM cultures (39 percent) were positive for dermatophytes. These authors concluded that DTM allows a higher recovery rate for dermatophytes than Sabouraud glucose culture, along with its ability to inhibit growth of bacteria and saprophytic contaminants.<sup>7</sup>

The repeat cultures obtained in 137 patients suggest that negative results of traditional fungal culture are not completely reliable; 28 percent (38/137) of patients with initially negative laboratory and DTM cultures were found to be positive for a dermatophyte on repeat culture. One retest culture was a nondermatophyte. In 19 of the retests, the result agreed with a positive DTM culture from the same patient. In 18 retests, the paired DTM result was negative. Thus, in both the original cultures and the retested specimens, only about 11 percent of the specimens that were DTM negative were positive by fungal culture, perhaps reflecting the longer time available for growth in cultures at the central laboratory.

Regardless of the culture method, the diagnostic yield can be improved by obtaining a culture specimen from the subungual nail debris, rather than from the nail bed or nail plate.<sup>11,12</sup> The specimen should be obtained by trimming the nail back to reveal the nail bed and then scraping away the subungual debris with a curette, as proximally as the patient finds tolerable, as in the present study.

In our sample of patients with symptoms of onychomycosis, cultures were positive in 48 percent (528/1100). This figure supports the widely quoted statistic that culture-confirmed onychomycosis represents 50 percent of all nail disease, which originated with a study published in

1968,<sup>13</sup> but appears unchanged to the present day.<sup>5,14</sup> Estimates of the proportion of patients with clinical nail problems in whom a combination of the two standard techniques can confirm a diagnosis of onychomycosis range from about 60 percent to 65 percent<sup>15,16</sup> to as high as 80 percent.<sup>17</sup> In this study, 67 percent of patients had dermatophyte-confirmed onychomycosis by either DTM or central laboratory culture. Routine utilization of DTM culture in the office practice to confirm clinically suspect onychomycosis and acceptance of this diagnostic test by individual managed care organizations would be expected to reduce the number of patients with onychomycosis who have the infection but are not treated.

Onychomycosis should be a concern of the primary care physician, and not only because its prevalence in the community makes it likely to be encountered in daily practice. As many as 50 percent of people with onychomycosis do not receive, nor do not seek, medical treatment, but medically confirmed onychomycosis should be treated.<sup>18</sup> Recent research has demonstrated the previously underestimated morbidity associated with onychomycosis. Extensive toenail infections can be painful, leading to difficulty standing or walking, limitations on wearing shoes, and consequent limitation of physical activity.<sup>19</sup> Effects on overall function and quality of life include reduced mobility and social activity in the elderly, reduced participation in leisure activities, and embarrassment or self-consciousness in social situations.<sup>19-21</sup> In diabetes patients, onychomycosis was associated with a threefold risk in secondary bacterial infections, such as erysipelas, gangrene, and foot ulcers.<sup>22</sup> At least one article in a recent managed care journal has stressed the need to confirm a diagnosis of onychomycosis using a simple, cost-effective procedure.<sup>23</sup>

The in-office DTM culture offers a simplified diagnosis of dermatophyte

infection that does not require knowledge of fungal colony morphology. The primary care practitioner can obtain nail-bed specimens and use the DTM culture method to confirm a presumptive diagnosis of onychomycosis in a large proportion of cases, with results available before infection can be confirmed by laboratory mycologic culture.

Not only does DTM offer a rapid and accurate diagnosis of dermatophyte infection, but it does so at a cost far less than traditional fungal culturing. Costs of traditional culturing in a hospital or independent laboratory may be as high as \$100, while the cost to the practitioner of the DTM medium is approximately \$1 per test. Other costs involved in performance of the DTM culture are trivial. In addition, if the practitioner performs the DTM culture in the office, reimbursement from most third-party insurance payers is available. Of more importance, if DTM cultures were routinely performed on all patients suspected to have fungal infections, a more rapid and accurate diagnosis could be made, with the potential to reduce the overall costs of care for these patients.

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