

Dermatophyte Test Medium Culture for Evaluating Toenail Infections in Patients With Diabetes

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OBJECTIVE — To evaluate the performance of the in-office dermatophyte test medium (DTM) culture when used to confirm the diagnosis of onychomycosis in diabetic patients.

RESEARCH DESIGN AND METHODS — Nail samples from 184 diabetic patients who exhibited symptoms consistent with toenail onychomycosis were screened for dermatophyte fungal infection using DTM, potassium hydroxide evaluation, and central mycology laboratory culture tests. The diabetic patient group investigated in this study is a subset of a heterogeneous set of patients who participated in a nationwide survey designed to investigate the use of fungal culture tests by dermatologists, podiatrists, and primary care physicians described in detail elsewhere. The overall sensitivity of the DTM and central laboratory culture methods was estimated and compared. Sensitivity differences between DTM and central laboratory culture methods were tested for statistical significance using the McNemar statistic.

RESULTS — DTM culture was positive in 102 of 184 patients (55%), while the central laboratory culture test detected the existence of fungal infection in 78 of 184 (42%). The two tests were in agreement (both positive or both negative) in 114 of 184 patients (62%). Central laboratory culture identified dermatophytes as the pathogen in 91% of positive cases.

CONCLUSIONS — DTM is a convenient and inexpensive culture test that can be used to confirm dermatophyte infections in diabetic patients with presumed onychomycosis. We found this test to be well suited for use in the primary care setting. Because oral antifungal agents are effective against dermatophyte species, which cause the vast majority of nail infections, diagnosis of onychomycosis requires confirmation of dermatophyte infection only, not identification of genus and species. DTM fulfills this requirement and has a diagnostic yield comparable to central laboratory culture.

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Onychomycosis, a condition that occurs with increased frequency in patients with diabetes (1–3), is known to exacerbate diabetic foot problems (4–7) and reduce patients' overall

quality of life (6,8–10). The dermatophyte fungi *Trichophyton rubrum* and *T. mentagrophytes* are the predominant pathogens, causing 80–90% of all onychomycosis infections (11–13). The re-

cent development of safe and effective orally administered agents active against dermatophyte fungi has significantly increased the cure rates for onychomycosis. The newer agents, terbinafine and itraconazole, have been associated with cure rates of 75% or higher in clinical trials in patients with laboratory-confirmed dermatophyte onychomycosis (14–16). When compared with the previous generation of systemic agents, which required long treatment durations and were ineffective in many patients, the newer agents represent a significant treatment improvement.

While it is possible to treat onychomycosis presumptively based on the clinical appearance of the nails, in the diabetic patient the diagnosis should be confirmed by culture to avoid treating conditions different from onychomycosis that can also produce abnormal-appearing nails. The traditional evaluation of onychomycosis consists of direct microscopic examination of potassium hydroxide (KOH)-treated nail specimens to confirm the presence of septate fungal hyphae, followed by fungal culture to identify the causative organism (17). These tests are limited in sensitivity and specificity (18) and are difficult to perform by nondermatologists who, in most cases, send nail samples to a reference laboratory for KOH and culture determinations. The tests are relatively costly, and waiting for etiologic information can delay treatment by 4–6 weeks.

The dermatophyte test medium (DTM) is an alternative culture method that can be used to confirm a diagnosis of onychomycosis. Although it does not identify specific organisms, DTM culture does confirm the presence of dermatophytes. The culture medium was originally described by Taplin et al. (19) as a test for the presence of dermatophytic molds. DTM is less expensive than fungal culture at a central laboratory, and its results are available much sooner, usually within 3–7 days. Dermatophyte growth is indicated by a change in the color of the DTM from yellow to red in response to

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Abbreviations: DTM, dermatophyte test medium; KOH, potassium hydroxide.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.

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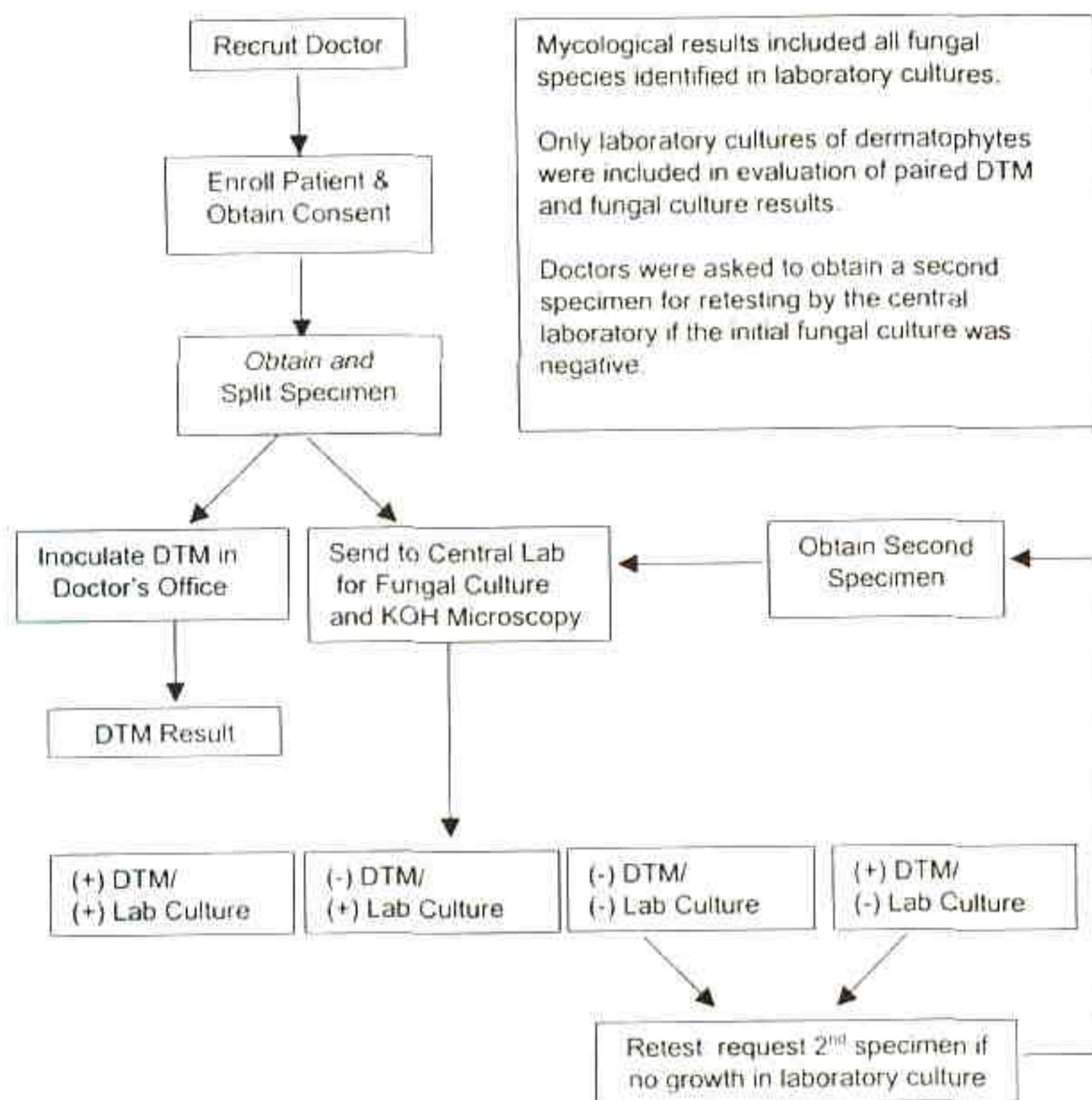


Figure 1—Infection confirmation survey design and procedures.

alkaline metabolites that result from growth of dermatophytes (19). The majority of DTM cultures can be identified within 1 week, and <2% of cultures require 2 weeks to show a change in color (19). The DTM contains gentamicin and chlorotetracycline to inhibit bacterial growth and cycloheximide to inhibit growth of saprophytic fungi. Although it does not identify specific organisms, a positive DTM culture confirms the presence of dermatophyte pathogens, which account for the vast majority of cases of onychomycosis (12,17). Taplin et al. correctly identified dermatophytes by DTM color change alone in 97% of 1,400 fungal cultures evaluated. DTM culture systems, suitable for use in the general practice office setting, are commercially available.

Prompt diagnosis and treatment of onychomycosis is particularly important in the diabetic patient. Sensory neuropathy, impaired circulation, and impaired immunological function render patients with diabetes at high risk for onychomycosis, while reduced vision and sensation may result in delays of its diagnosis. In the diabetic patient, onychomycosis increases the risk of foot ulcer, cellulitis,

and gangrene, as well as osteomyelitis (20).

The present analysis evaluates the use of DTM culture in a set of patients with diabetes who were enrolled in a large nationwide study by office-based primary care physicians, dermatologists, and podiatrists. The study compared office-based DTM with central laboratory fungal culture in patients exhibiting signs and symptoms of onychomycosis.

RESEARCH DESIGN AND METHODS

Patients were enrolled in the study by office-based primary care physicians, dermatologists, and podiatrists. The physician sample was stratified by specialty and region to ensure a uniform representation of primary care physicians, dermatologists, and podiatrists throughout the U.S. Each physician was asked to enroll five or more patients with signs and symptoms of onychomycosis. All patients were ≥ 18 years of age. Physicians were specifically not asked to include patients with diabetes; rather, patients were enrolled regardless of their comorbid disease status. Patients were excluded from the study if they had received

oral antifungal therapy within the previous 90 days and any topical antifungal agent within the previous 30 days. Patient enrollment was initiated on 1 July 2000, and data collection was completed 9 November 2001.

At the initial office visit, the physician explained the nature of the study, obtained written informed consent, and collected demographic information as well as medical history. The physician then obtained a specimen from the toenail bed for mycological evaluation. A portion of the specimen was used for DTM evaluation (ACU-DTM; Accuderm, Ft. Lauderdale, FL), which was performed in the office, while the remaining portion of the specimen was sent to a central laboratory for KOH testing and fungal culture. The University of Texas Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio was used for that purpose.

Results of DTM evaluations were available within 2 weeks, whereas the central laboratory culture results were available ~4–6 weeks after the sample was obtained. The study design is illustrated in Fig. 1. The primary analyses conducted in this study were 1) a comparison of the results of central laboratory and DTM cultures and 2) a tabulation of the infectious organisms identified by fungal culture. The Western Institutional Review Board (Olympia, WA) approved the study protocol and all required materials.

Mycological evaluations

Physicians were supplied with DTM kits and a brief video describing how to obtain a nail bed sample. 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. Small pieces of the subungual debris were sampled from the proximal nail bed with a probe or curette. Immediately after the specimen was obtained, it was divided. One part was placed on the DTM medium and incubated at room temperature for up to 2 weeks according to the instructions provided by the DTM kit manufacturer. The remainder of the specimen was sent to the central laboratory for KOH testing and fungal culture.

The central laboratory performed its evaluations using well-established methods. Fungal culture was carried out using two media: one contained cycloheximide

to inhibit nondermatophyte pathogens, while the other contained Sabouraud dextrose agar to allow the growth of yeasts and nondermatophyte fungi known to cause onychomycosis.

Statistical analysis

Because this is a descriptive study, data tabulation and summary statistics were the predominant methods of analysis. Agreement between the DTM and central laboratory culture methods was measured by using the κ statistic (21). The asymptotic SE and 95% confidence limits for κ were calculated using SAS software (22). The McNemar statistic was used to test the statistical significance of sensitivity differences between paired DTM and laboratory fungal culture results.

RESULTS— A total of 322 physicians participated in the survey, of which 310 provided data from diabetic patients. The physician/diabetic patient group consisted of 45 dermatologists (15%), 119 podiatrists (38%), and 146 primary care physicians (47%). The total number of patients enrolled in the survey was 1,343. All of the patients had signs and symptoms of onychomycosis; however, only 1,177 (88%) had complete datasets available by the deadline for data collection and therefore were the only ones used in the survey. (Interim results from 670 patients whose data were available after the first phase of the survey was completed have been reported elsewhere [23].) Of those with complete datasets, 184 of 1,177 (16%) were diabetic. The diabetic group consisted of 129 Caucasians (70%), 30 African Americans (16%), 22 Hispanics (12%), 1 Asian (1%), and 2 from other racial groups (1%). The majority of diabetic patients were men (61%) between 55 and 64 years of age (57%). In addition to the toenail onychomycosis, fingernail onychomycosis and clinical tinea pedis were identified in 8 and 33% of diabetic patients, respectively.

Central laboratory culture identified specific pathogens in 78 (42%) of the 184 patients. The in-office DTM culture results were positive in 102 of these 184 patients (55%). Dermatophyte organisms were the predominant pathogens. Dermatophytes were isolated from 71 (91%) of the 78 patients with a positive culture (Table 1). Nondermatophyte organisms were isolated in cultures from eight patients: *Scopulariopsis brevicaulis* in four pa-

Table 1—Pathogens identified by central laboratory culture in the 78 patients with diabetes, onychomycosis symptoms, and positive fungal cultures

Organism	n (%) of patients
Dermatophytes	
<i>T. rubrum</i>	63 (80.8)
<i>T. mentagrophytes</i>	7 (9.0)
<i>E. floccosum</i>	1 (1.3)
Nondermatophyte molds	
<i>S. brevicaulis</i>	4 (5.1)
All others	4 (5.1)

The number of patients listed totals 79 because the sample from one patient tested positive for *T. rubrum* and *S. brevicaulis*. This also explains why the sum of the percent values shown is 101.3%.

tients and *Scytalidium dimidiatum*, *Aspergillus terreus*, *Fusarium* spp., and *Alternaria* spp. in one patient each. The number of dermatophyte- and nondermatophyte-positive cultures exceeds the number of patients (79 instead of 78) because the sample from one patient tested positive for two types of organisms. Overall, the incidence rates observed in the diabetic sample closely match the rates observed in the heterogeneous patient group ($n = 1,177$), for which dermatophytes were the causative organism in 92% of positive cultures and nondermatophytes accounted for the remaining 8%.

The results of central laboratory and DTM cultures were compared in the 184 diabetic patients for whom data from both tests were available. The two tests were in agreement in 114 (62%) of the samples (i.e., both positive or both negative). Detailed results are shown in Table 2. The calculated κ was 0.25 (95% CI 0.18–0.39), indicating fair agreement beyond that expected by chance (20).

Table 2—Comparison of in-office DTM culture and central laboratory mycological culture in 184 subjects with diabetes

		Central laboratory culture		Total
		+	–	
DTM	+	55 (29.9%)	47 (25.5%)	102 (55.4%)
	–	23 (12.5%)	59 (32.1%)	82 (44.6%)
	Total	78 (42.4%)	106 (57.6%)	184 (100%)

A positive result (+) is defined as fungal growth in central laboratory culture or a color change in DTM culture. A negative result (–) is defined as no growth in fungal culture or lack of color change by 2 weeks in DTM culture.

In cases where the results of the two culture methods were in disagreement, laboratory culture was negative twice as often as DTM culture (47 vs. 23 patients) (Table 2). Central laboratory cultures were negative in 106 of the 184 patients (58%) with paired culture results. A second specimen was requested from these patients for retesting, and laboratory results were available for 41 patients at the time of data analysis. The retest culture was positive in seven patients (17% of the patients who were retested).

CONCLUSIONS— Analysis of this nationwide survey of patients with diabetes and symptoms of onychomycosis indicates a fair degree of agreement between the culture methods evaluated ($\kappa = 0.25$), with DTM more likely to yield positive results than central laboratory culture, as indicated by McNemar's χ^2 test ($P = 0.004$). The frequency of successful culture, 55.4% for DTM and 42.4% for central laboratory culture, may be a reflection of the well-known difficulties of culturing the slow-growing organisms responsible for nail fungal infections. The rate of agreement of these two culture methods in diabetic patients may also be affected by a hesitancy of physicians to sample aggressively the subungual nail debris due to fear of inflicting injury. Subsequent division of each specimen for multiple evaluations increases the likelihood of obtaining inadequate material for all required tests, resulting in false negative results. In addition, results of either culture method can be negative, even when KOH microscopy is positive, if the specimen is inadequate, the patient has used a topical agent that suppressed fungal growth but did not eliminate the pathogen, or bacterial or fungal contaminants inhibited the growth of dermatophytes in culture. A criticism of DTM

culture is that nondermatophyte contaminants may cause false positive results (24). In the present study, nondermatophytes were identified as the sole organism in 7 of 78 culture specimens (9.0%), of which 3 were positive by DTM. (Non-dermatophyte organisms resulting in a false positive DTM test were *Fusarium* spp., *S. dimidiatum*, and *S. bicucullis*.)

Onychomycosis is clearly a concern in patients with diabetes. The prevalence of fungal nail infections is elevated in patients with diabetes compared with estimated background rates of 2–13% (24). Gupta et al. (1) identified onychomycosis by mycological testing in 26% of 550 North American patients with type 1 or type 2 diabetes attending either diabetes clinics or dermatologists' offices. The prevalence of onychomycosis in that sample was nearly three times higher than in a comparison group of nondiabetic dermatology patients after adjustment for age and sex. That study excluded patients with a referring diagnosis of onychomycosis; thus, the investigators estimated that the true prevalence of toenail onychomycosis in patients with diabetes might be as high as one-third. In another large multicenter study, diabetes was identified as a risk factor associated with a twofold elevation in onychomycosis over background levels (2). An analysis of a large managed care database found that nearly 6% of patients with diabetes filed a claim for treatment of onychomycosis in a 2-year period, as compared with 0.8% of patients without diabetes (3).

Onychomycosis contributes to the severity of diabetic foot problems (4–7). Sharp, brittle nails can gouge the skin, creating a portal for entry of bacterial organisms. Onychomycosis is often associated with tinea pedis, which can create fissures in the skin, again opening the way for bacterial infections. Thickened mycotic nails can cause pressure necrosis of the nail bed. These injuries may go unnoticed in patients with impaired sensation due to peripheral neuropathy. Because of the proximity of the nail bed to underlying bone, osteomyelitis can develop as a consequence of neglected nail bed erosion (7). In a retrospective study of outpatient medical care claims, onychomycosis was associated with a threefold increase in gangrene and foot ulcers among patients with diabetes (3). The presence of onychomycosis can contribute to the difficulties of maintaining nail

hygiene in elderly diabetic patients. Onychomycosis can also contribute to self-consciousness, lowering of self-esteem, and restriction of social activities in younger diabetic individuals (6).

The prevalence of dermatophyte infection among culture-positive patients in the present study, 91%, is similar to that in the large North American clinic-based diabetic patient population (1). Dermatophytes have also represented the large majority of confirmed infections in large-scale epidemiological studies of heterogeneous patient populations (2,12,26,27). *Candida* species were isolated in 3 of the 110 (3%) culture-positive diabetic patients studied by Gupta et al. (1) but were not isolated in any of the fungal cultures in this study.

Dermatophyte fungi cause the overwhelming majority of toenail infections, and the available oral therapies are active against all dermatophyte species. Therefore, in the majority of patients, diagnosis does not require identification of the genus or species of the pathogen, only determination of the presence or absence of dermatophytes. Laboratory fungal culture, together with KOH microscopy, is the traditional method for confirming a clinical diagnosis of toenail onychomycosis. As an alternative to lab culture, DTM culture is rapid, inexpensive, and easily performed in the primary care office. The cost of DTM culture in this study was ~\$1.00 per test compared with \$25.00 for each fungal culture. DTM and KOH evaluation can confirm a presumptive diagnosis, with results available before infection is confirmed by laboratory culture. Traditional laboratory culture may be offered as a secondary diagnostic test if KOH microscopy indicates septate hyphae, DTM culture is negative, and the appearance of the nail is strongly suggestive of onychomycosis. Histopathologic examination with periodic acid Schiff stain is an alternative to KOH microscopy that is becoming more widely used to demonstrate fungal elements (28). As with KOH, however, periodic acid Schiff stain does not demonstrate the presence of viable yeasts, fungi, or molds. Once identified, onychomycosis may be readily treated with the available oral agents, which, as recent clinical studies and post-marketing surveys have shown (29–31), are well tolerated by patients with diabetes. The manufacturer's prescribing information should be followed with regard to

monitoring of hepatic function during treatment and warnings against use in patients with preexisting renal, liver, or heart failure. To our knowledge, this is the largest study to evaluate the agreement of DTM culture and central laboratory fungal culture for confirmation of a clinical diagnosis of onychomycosis in diabetic patients, as well as one of the largest to describe the prevalence of onychomycosis pathogens in this patient population.

References

- Gupta AK, Konnikov N, MacDonald P, Rich P, Rodger NW, Edmonds MW, McManus R, Summerbell RC: Prevalence and epidemiology of toenail onychomycosis in diabetic subjects: a multicentre survey. *Br J Dermatol* 139:665–671, 1998
- Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, Sullivan S, Daniel R, Krusinski P, Fleckman P, Rich P, Odom R, Aly R, Pariser D, Zaiac M, Rebell G, Lesher J, Gerlach B, Ponce-De-Leon GF, Ghannoum A, Warner J, Isham N, Elewski B: A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J Am Acad Dermatol* 43:641–648, 2000
- Boyko WL, Doyle JJ, Ryu S, Gause D: Onychomycosis and its impact on secondary infection development in the diabetic population (Poster). Presented at the 4th annual meeting of the International Society for Pharmacoeconomics and Outcomes Research (ISPOR), Arlington, VA, 23–26 May 1999
- Greene RA, Scher RK: Nail changes associated with diabetes mellitus. *J Am Acad Dermatol* 16:1015–1021, 1987
- Levy LA: Epidemiology of onychomycosis in special-risk populations. *J Am Podiatr Med Assoc* 87:546–550, 1997
- Rich P: Special patient populations: onychomycosis in the diabetic patient. *J Am Acad Dermatol* 35:S10–S12, 1996
- Rich P, Hare A: Onychomycosis in a special patient population: focus on the diabetic. *Int J Dermatol* 38 (Suppl. 2):17–19, 1999
- Drake LA, Scher RK, Smith EB, Faich GA, Smith SL, Hong JJ, Stiller MJ: Effect of onychomycosis on quality of life. *J Am Acad Dermatol* 38:702–704, 1998
- Lubeck DP: Measuring health-related quality of life in onychomycosis. *J Am Acad Dermatol* 38:S64–S68, 1998
- Schein JR, Gause D, Stier DM, Lubeck DP, Bates MM, Fisk R: Onychomycosis: baseline results of an observational study. *J Am*

- Podiatr Med Assoc* 87:512–519, 1997
11. Evans EG: Causative pathogens in onychomycosis and the possibility of treatment resistance: a review. *J Am Acad Dermatol* 38:532–536, 1998
 12. Kemna ME, Elewski BE: A U.S. epidemiologic survey of superficial fungal diseases. *J Am Acad Dermatol* 35:539–542, 1996
 13. Summerbell RC: Epidemiology and ecology of onychomycosis. *Dermatology* 194 (Suppl. 1):32–36, 1997
 14. Elewski BE: Onychomycosis: pathogenesis, diagnosis, and management. *Clin Microbiol Rev* 11:415–429, 1998
 15. Gupta AK, Shear NH: The new oral antifungal agents for onychomycosis of the toenails. *J Eur Acad Derm Venereol* 13:1–13, 1999
 16. Niewerth M, Korting HC: Management of onychomycoses. *Drugs* 58:283–296, 1999
 17. Elewski BE: Diagnostic techniques for confirming onychomycosis. *J Am Acad Dermatol* 35:S6–S9, 1996
 18. Pierard GE, Arrese JE, De Doncker P, Pierard-Franchimont C: Present and potential diagnostic techniques in onychomycosis. *J Am Acad Dermatol* 34:273–277, 1996
 19. Taplin D, Zaias N, Rebell G, Blank H: Isolation and recognition of dermatophytes on a new medium (DTM). *Arch Dermatol* 99:203–209, 1969
 20. Gupta AK, Humke S: The prevalence and management of onychomycosis in diabetic patients. *Eur J Dermatol* 10:379–384, 2000
 21. Feinstein AR: *Clinometrics*. New Haven, CT, Yale University Press, 1987
 22. Stokes ME, Davis CE, Koch GG: *Categorical Data Analysis Using the SAS Software*, 2nd ed. Cary, NC, SAS Institute, 2000
 23. Daniel CR III, Elewski B: The diagnosis of nail fungus infection, revisited. *Arch Dermatol* 136:1162–1164, 2000
 24. Elewski BE, Leyden J, Rinaldi M, Atillasoy E: Office-based confirmation of onychomycosis: a U.S. prospective survey. *Arch Intern Med* 162:2133–2138, 2002
 25. Albreski DA, Gupta AK, Gross EG: Onychomycosis in diabetes: management considerations (Review). *Postgrad Med (Spec. no.):*26–30, 1999
 26. Elewski BE: Large-scale epidemiological study of the causal agents of onychomycosis: mycological findings from the Multicenter Onychomycosis Study of Terbinafine (Letter). *Arch Dermatol* 133:1317–1318, 1997
 27. Gupta AK, Jain HC, Lynde CW, MacDonald P, Cooper EA, Summerbell RC: Prevalence and epidemiology of onychomycosis in patients visiting physicians' offices: a multicenter Canadian survey of 15,000 patients. *J Am Acad Dermatol* 43:244–248, 2000
 28. Harvey CK, Richardson A: Techniques for obtaining specimens for culture to confirm onychomycosis. *J Am Podiatr Assoc* 90:394–396, 2000
 29. Farkas B, Paul C, Dobozy A, Hunyadi J, Horvath A, Fekete G: Terbinafine (Lamisil) treatment of toenail onychomycosis in patients with insulin-dependent and non-insulin-dependent diabetes mellitus: a multicentre trial. *Br J Dermatol* 146:254–260, 2002
 30. Hall M, Monka C, Krupp P, O'Sullivan D: Safety of oral terbinafine: results of a post-marketing surveillance study in 25,884 patients. *Arch Dermatol* 133:1213–1219, 1997
 31. Verspeelt J, Marynissen G, Gupta AK, De Doncker P: Safety of itraconazole in diabetic patients. *Dermatology* 198:382–384, 1999