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Repetitive-Sequence-PCR-Based DNA Fingerprinting Using the DiversiLab System for Identification of Commonly Encountered Dermatophytes

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The performance of repetitive-sequence-based PCR (rep-PCR) using the DiversiLab system for identification of dermatophytes commonly isolated in a clinical laboratory was assessed by comparing results to those of conventional tests (colony morphology, microscopic examination of slide cultures, and, for suspected *Trichophyton* species, use of additional media). Sixty-one cultures were tested in phase 1, the feasibility portion of the study; 64 additional cultures were tested in phase 2, the validation portion conducted to assess reproducibility and confirm accuracy. Discrepancies were resolved by repeating rep-PCR and conventional tests and, in phase 2, sequencing the internal transcribed spacers. After initial testing of the cultures in phase 1 (excluding one contaminated culture), agreement between conventional tests and rep-PCR was 90% (54 of 60). Agreement was 98.3% after resolution of discrepancies, and in all but one case the initial rep-PCR result was correct. After initial testing of cultures in phase 2 (excluding one discarded and one contaminated culture), agreement between rep-PCR and conventional testing was 88.7% (55 of 62). After discrepancies were resolved, agreement was 100%. Initial rep-PCR results were correct, except for one *Microsporum canis* culture containing two colony variants, which could not be initially identified by rep-PCR. The performance of the DiversiLab system for identification of the dermatophytes commonly encountered in a clinical mycology laboratory—*Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *M. canis*—was excellent. Moreover, the DiversiLab system is technically simple and provides results in <24 h once a pure culture is available for testing, which is considerably more rapid than conventional identification tests.

Dermatophytes are keratinophilic fungi that cause infections of skin, hair, and nails. The three genera of dermatophytes generally are identified in the laboratory based on colony morphology and the microscopic appearance of conidia (8, 10). *Trichophyton* species have numerous microconidia and rare thin-walled smooth macroconidia; *Microsporum* species have many rough, thick-walled macroconidia, and microconidia usually are also present; *Epidermophyton floccosum* has numerous thin- and thick-walled smooth macroconidia but no microconidia. Identification of *Trichophyton* to the species level also may require inoculation of urea and *Trichophyton* agars. Slide cultures and use of special media is time-consuming; results often are not available for several weeks. Additionally, relying on phenotypic features for identification occasionally is problematic because the distinguishing characteristics of these fungi are not stable (1).

The DiversiLab system (Spectral Genomics, Houston, TX) is a rapid, technically simple method that uses repetitive-sequence-based PCR (rep-PCR) to determine relatedness of many organisms and to identify *Aspergillus* and *Candida* to the species level (2, 7). This system has three components: rep-PCR reagent kits; the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA), which separates the amplified fragments on a microfluidic chip and detects them based on fluorescent intensity and migration time; and the DiversiLab web-based software. The software analyzes results by creating a proximity matrix using the Pearson correlation to calculate pair-wise similarities between all samples tested. The report generated by the DiversiLab system includes a dendrogram (which illustrates pair-wise similarity relationships among isolates) and scatter plot (which provides a spatial, nonhierarchical view of the relationships); gel-like images, an electropherogram, or both can also be incorporated. Commercially available components, automation, technical simplicity, rapid turnaround time, and user-friendly reports are features that make the DiversiLab system attractive for a busy microbiology laboratory. The objective of this study was to assess the reliability of the DiversiLab system for identification of dermatophytes commonly encountered in a clinical mycology laboratory to the species level.

MATERIALS AND METHODS

Study design. This study was conducted in two phases. First, the feasibility of using rep-PCR as a method to identify dermatophytes was investigated. In this phase, the rep-PCR procedure was optimized at Spectral Genomics, Inc. Second, the reproducibility and accuracy of the test were evaluated at ARUP. To assess reproducibility, a subset of isolates was tested on separate days.

Isolates. All patient information associated with the isolates in this study was removed. In phase 1, clinical dermatophyte isolates, previously identified at ARUP by using conventional methods (see below), were selected for testing by rep-PCR and mailed in two groups to Spectral Genomics, Inc. The first group

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included 41 cultures, each labeled with the species name (initially identified as *Trichophyton rubrum* [$n = 8$], *Trichophyton mentagrophytes* [$n = 9$], *Trichophyton tonsurans* [$n = 11$], *Microsporum canis* [$n = 9$], *Microsporum gypseum* [$n = 2$], *E. floccosum* [$n = 1$], and *Microsporum nanum* [$n = 1$]). The second group included 20 cultures that were assigned arbitrary code numbers (3 *T. rubrum*, 4 *T. mentagrophytes*, 4 *T. tonsurans*, 7 *M. canis*, and 2 *M. gypseum*); the species name was not provided until rep-PCR testing was completed. In phase 2, to assess reproducibility, each of 10 previously identified isolates (3 *T. tonsurans*, 4 *T. rubrum*, 1 *T. mentagrophytes*, 1 *Trichophyton verrucosum*, and 1 *M. gypseum*) was subcultured to three potato dextrose agar (PDA) slants, each of which was assigned an arbitrary code number. Thirty-four additional coded isolates were tested to assess accuracy (initially identified as *T. rubrum* [$n = 11$], *T. mentagrophytes* [$n = 10$], *T. tonsurans* [$n = 7$], and *M. canis* [$n = 6$]).

Conventional identification. Identification of dermatophytes was based on colony morphology and microscopic examination of slide cultures (8, 10). For suspected *Trichophyton* species, urea and *Trichophyton* agars no. 1 and no. 4 also were inoculated. After identification, cultures were stored in sterile water at room temperature.

Rep-PCR. Personnel performing rep-PCR were blinded to the results of conventional testing (except in the initial part of the feasibility phase as described above). All work was performed in a biosafety level 2 biological safety cabinet until cells were lysed. Fungal samples were grown on PDA slants at 30°C for 5 to 7 days. DNA was extracted from a spore and mycelial mass (a minimum of 2 square centimeters) by using an UltraClean Microbial DNA isolation kit (Mo Bio Laboratories, Solana Beach, CA) in accordance with the manufacturer's instructions with the following modifications. Prior to extraction, a freezing (−80°C for 30 min) step and a heating (80°C for 30 min) step were added, and the bead beating step was extended from 10 to 30 min. The fungal DNA was amplified using a DiversiLab Mould Kit (Spectral Genomics, Inc.) for DNA fingerprinting in accordance with the manufacturer's instructions. Briefly, 2 μl of genomic DNA was added to the rep-PCR master mix with fungal specific primers, 2.5 U AmpliTaq, and 10× PCR buffer (Applied Biosystems, Inc., Foster City, CA) for a 25-μl total reaction. Thermal cycling parameters were as follows: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 70°C for 90 s, and a final extension at 70°C for 3 min. Detection and analysis of rep-PCR products were implemented using the DiversiLab system, in which the amplified fragments of various sizes and fluorescence intensities were separated and detected using a microfluidics chip with an Agilent 2100 bioanalyzer. Further analysis was performed with the web-based DiversiLab software, version 2.1.66, which uses the Pearson correlation coefficient and unweighted pair group method with arithmetic mean to automatically compare the rep-PCR-based DNA fingerprints of unknown isolates. All bands in the gel-like image are considered in the analysis, although more intense bands are weighted more heavily.

Species identification of the fungal cultures was based on the percentage similarity with and clustering profile obtained from the dendrograms, as well as a visual comparison of the virtual gel images. After analysis of the phase 1 data, guidelines for identification to the species level were developed. A culture was given a species identification if the fingerprint pattern had ≥85% similarity to a single fungus or a cluster of fungi in the database, which includes the dermatophytes tested in this study, several species of *Aspergillus*, *Zygomycetes*, *Fusarium* species, *Penicillium* species, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*. If there was <85% similarity, the culture could not be identified to the species level.

Resolution of discrepancies. In both phases of the study, when rep-PCR and conventional test results did not agree, both methods were repeated. Personnel who performed the conventional tests did so without knowledge of the rep-PCR results, and for rep-PCR, DNA was reextracted from cultures. Additionally, in phase 2, sequencing of the internal transcribed spacers (ITS1-5.8S-ITS2) was performed (3–6, 11). Briefly, DNA prepared for rep-PCR was amplified using the primers ITS1 forward 5′TCCGTAGGTGAACCTGCGG3′ and ITS4 reverse 5′TCCTCCGCTTATTGATATGC3′ (11). Three microliters of template DNA was added to 1× LightCycler FastStart DNA Master Hybridization Probes mixture (Roche Applied Science, Penzberg, Germany), which contains deoxynucleoside triphosphates, FastStart Taq DNA polymerase, and 1 mM $MgCl_2$. An additional 3.6 μl of 25 mM $MgCl_2$ (final $MgCl_2$ concentration, 4.6 mM), 0.4 μM concentrations of the primers, and 1× SYBR green (Molecular Probes, Inc., Eugene, OR) were added for a total volume of 25 μl. Thermal cycling parameters using a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) were as follows: polymerase activation at 95°C for 10 min, 50 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 20 s, extension at 76°C for 30 s with fluorescence acquisition during each cycle, and a final extension at 72°C for 2 min. A melting curve analysis from 55°C to 99°C followed the amplification. The amplified product was processed for sequencing using ExoSAP-IT (USB Corp., Cleveland, OH).

Bidirectional DNA sequence data were generated for each dermatophyte sample using fluorescently labeled terminator sequencing chemistry and sequencing primers (5′ ITS1 primer and 3′ ITS4 primer). Five microliters of BigDye Terminator Ready Reaction Mix v. 1.1 (Applied Biosystems) was added to 4 μl of each primer (0.8 pmol/μl); 3 μl of purified PCR product then was added to the BigDye-primer mix. Cycle sequencing was performed using a 9700 Thermal Cycler (Applied Biosystems) with the following parameters: 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reaction products were passed through Sephadex G-50 Fine column to remove unincorporated dye terminators. DNA sequencing data files from the purified sequencing reaction products were generated using an ABI Prism 3730 DNA analyzer (Applied Biosystems, Inc). By using DNASTAR sequencing analysis software v. 5.06 (DNASTAR, Inc., Madison, WI), 5′ and 3′ sequencing files were aligned and edited to create a consensus sequence for each dermatophyte sample. The consensus sequence was then compared to all sequences in NCBI GenBank v. BLAST 2.2 10.

RESULTS

Phase 1. After initial testing of the first 41 isolates, a dendrogram illustrated that all 8 *T. rubrum* samples grouped together, as did the 11 *T. tonsurans* and 9 *M. canis* samples. There were two clusters of *T. mentagrophytes*, each with three isolates. Percentage similarity thresholds were determined to be ≥85%, based on the outermost similarity of each of these clusters. Of the remaining three cultures initially identified as *T. mentagrophytes* by conventional tests, one (Fig. 1, sample no. 10) grouped with *T. rubrum*, one grouped with *T. tonsurans* (Fig. 1, no. 30), and one could not be identified (its fingerprint pattern had less than 85% similarity to the other fungi in the database). One of the cultures initially identified by conventional tests as *M. gypseum* (Fig. 1, no. 58) grouped with the *E. floccosum*. The culture initially identified as *M. nanum* had a fingerprint pattern with less than 85% similarity to the other dermatophytes in the database (Fig. 1, no. 19). This was the only *M. nanum* isolate analyzed in this study.

Retesting isolates with discrepant rep-PCR and conventional test results showed that the initial rep-PCR results were correct (Table 1). Initial and repeat rep-PCR results were the same, whereas the repeat conventional test results changed. The sample identified as *T. mentagrophytes* (by conventional testing), with a rep-PCR fingerprint pattern that had less than 85% similarity to the other fungi in the database, was contaminated. Therefore, this culture was excluded from the analysis. The sample identified as *T. mentagrophytes* (by conventional testing), which had initially grouped with *T. rubrum* by rep-PCR, was reidentified as a *T. rubrum* by both methods (Table 1, specimen no. 10), and the one that grouped with *T. tonsurans* by rep-PCR was reidentified as *T. tonsurans* by both methods (Table 1, no. 30). The *M. gypseum* (by conventional testing) that had grouped with the *E. floccosum* by rep-PCR was reidentified as *E. floccosum* by both methods (Table 1, no. 58).

The 20 coded cultures tested in the second part of phase 1 all clustered with isolates of their respective species, as illustrated in Fig. 1, with one exception. One *M. gypseum* had a rep-PCR fingerprint pattern that had less than 85% similarity to the other fungi in the database and, therefore, was not identified (Fig. 1 and Table 1, no. 56).

Phase 2. In the reproducibility portion of phase 2, rep-PCR and conventional test results agreed for all 30 cultures (10 different isolates, each subcultured to three PDA slants) with

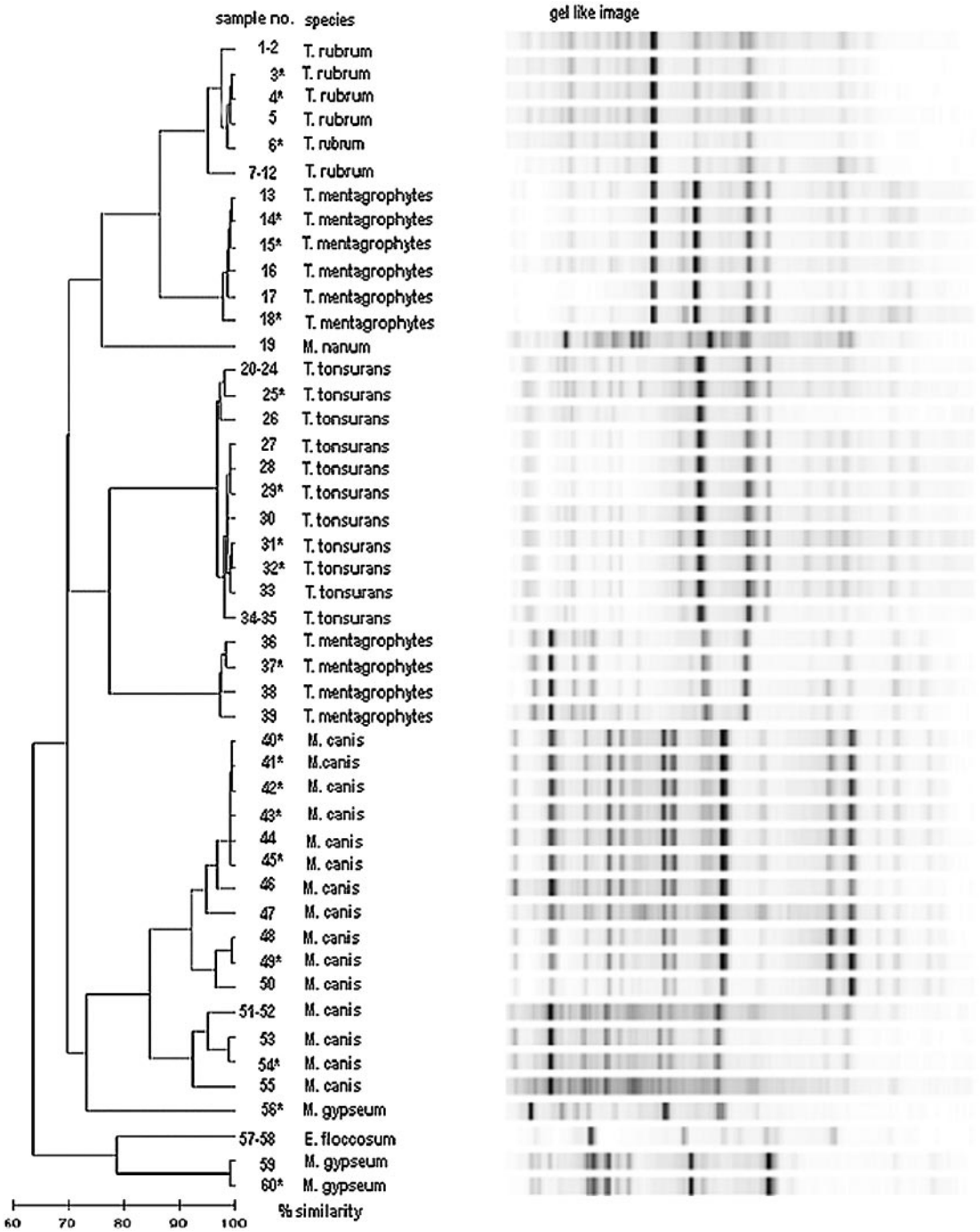

FIG. 1. Dendrogram of dermatophyte cultures tested at Spectral Genomics in Phase 1 of the study, excluding the contaminated culture. Gel-like images are created by the DiversiLab system. Coded cultures tested in the second part of phase 1 are marked with an asterisk. The horizontal bar at the bottom left of the dendrogram indicates the percent similarity coefficient within the species. Gel-like images of uncoded samples in the first part of phase 1 with ≥99% similarity were condensed. Coded cultures were not condensed.

one exception. One culture was identified as *T. tonsurans* by conventional tests but as *T. mentagrophytes* by rep-PCR. Unfortunately, this culture had inadvertently been discarded and repeat testing could not be performed. Therefore, because the discrepancy could not be resolved, it was excluded from the analysis.

After initial testing of the 34 additional cultures evaluated, rep-PCR and conventional test results agreed for 3 of 5 *M. canis* samples, 8 of 10 *T. mentagrophytes* samples, 6 of 7 *T. tonsurans* samples, and 9 of 12 *T. rubrum* samples. One culture initially identified as *T. rubrum* by conventional tests could not be identified by rep-PCR (the fingerprint pattern had less than 85% similarity to the other fungi in the database). The subculture of this sample prepared for retesting was overgrown with

TABLE 1. Comparison of rep-PCR and conventional test analyses for isolates in phase 1 with initially discrepant rep-PCR and conventional test results

Specimen no.	Result with:			
	Conventional testing		Rep-PCR[b]	
	Initial	Repeat	Initial	Repeat
10	*T. mentagrophytes*	*T. rubrum*	*T. rubrum*	*T. rubrum*
30	*T. mentagrophytes*	*T. tonsurans*	*T. tonsurans*	*T. tonsurans*
58	*M. gypseum*	*E. floccosum*	*E. floccosum*	*E. floccosum*
56[a]	*M. gypseum*	*M. gypseum*	No ID	No ID

[a] Sample 56 appears to be a *M. gypseum* with a fingerprint pattern that is different from the other 2 *M. gypseum* samples tested.
[b] No ID, isolate could not be identified.

bacteria and, therefore, was excluded from the analysis. Conventional and rep-PCR results for the remaining seven cultures with initially discrepant results are shown in Table 2. After the seven cultures with discrepant results were retested, agreement between rep-PCR and conventional testing was 100% (Fig. 2, Table 2). For rep-PCR, repeat and initial results were identical, with one exception. Close scrutiny of the culture that initially was identified as *M. canis* by conventional tests but could not be identified by rep-PCR showed two colony types. Each colony type was subcultured to a separate PDA slant and retested; both colony morphologies were identified as *M. canis* by rep-PCR and conventional tests (Fig. 2 and Table 2, nos. 89 and 90). For the remaining isolates with initially discrepant results, repeat conventional testing yielded a different identification. Two cultures did not sporulate when retested by conventional methods and, therefore, were called nonsporulating molds (Fig. 2 and Table 2, nos. 84 and 94). One (Fig. 2, no. 84) initially was identified as *M. canis* by conventional tests but as *T. rubrum* by rep-PCR. The other (Fig. 2, no. 94) was identified as *T. mentagrophytes* by conventional tests but was not identified by rep-PCR (it had a fingerprint pattern with less than 85% similarity to the other fungi in the database). The culture initially called *T. mentagrophytes* by conventional tests and *T. tonsurans* by rep-PCR was reidentified as *T. tonsurans* by both methods (Fig. 2, no. 70). The culture identified as *T. tonsurans* by conventional tests but *T. rubrum* by rep-PCR was reidentified by conventional tests as *T. rubrum* (Fig. 2, no. 80). The culture identified as *T. rubrum* by conventional tests but *T. tonsurans* by rep-PCR was reidentified as a *T. tonsurans* (Fig. 2 and Table 2, no. 74), and the culture identified as *T. mentagrophytes* by rep-PCR was reidentified as *T. mentagrophytes* (Fig. 2 and Table 2, no. 67).

For the cultures in phase 2 with initially discrepant rep-PCR and conventional test results, internal transcribed space (ITS) sequencing confirmed the rep-PCR identification (Table 2), with one potential exception. Specimen 94, which could not be identified by rep-PCR (because it had less than 85% similarity to other fungi in the database) or conventional tests (because it did not sporulate when reevaluated), was called *Microsporum audouinii* by ITS sequencing. *M. audouinii* was not in the Spectral Genomics database, which explains the failure of the system to provide an identification for that culture.

DISCUSSION

Dermatophytes comprise a substantial proportion of the fungal cultures identified in a clinical mycology laboratory. At ARUP, one-third to one-half of the fungal cultures undergoing testing at any one time are dermatophytes. Identification of dermatophytes usually is straightforward, but the morphological characteristics of the different species are not always stable and occasional cultures do not sporulate after incubation for several weeks on different media. Additionally, when using conventional tests for identification, e.g., slide culture and, for suspected *Trichophyton* species, urea and *Trichophyton* agars, results are not reported for up to 3 weeks after growth is apparent (8, 10). Rep-PCR using the automated DiversiLab system recently was shown to provide rapid and accurate identification of the frequently encountered *Aspergillus* species (7). The goal of this study was to assess the ability of the DiversiLab system to accurately and reproducibly identify the common dermatophytes.

We found that the performance of rep-PCR for identification of dermatophyte species commonly isolated in a clinical laboratory was excellent. Of the cultures in the analysis of the phase 1 data, agreement between conventional testing and rep-PCR was 90% (54 of 60) after initial testing. After resolution of discrepancies, agreement was 98.3% (59 of 60), and in all but one case, the initial rep-PCR result was correct. One *M. gypseum* sample (Table 1 and Fig. 1, no. 56) had <85% similarity to other fungi in the database, including the other two *M.*

TABLE 2. Comparison of ITS sequencing, rep-PCR, and conventional test analyses for isolates in phase 2 with initially discrepant rep-PCR and conventional test results

Specimen no.	Result with:				ITS sequencing identification
	Conventional testing		Rep-PCR[b]		
	Initial	Repeat	Initial	Repeat	
94	*T. mentagrophytes*	Nonsporulating mold	No ID	No ID	*M. audouinii*
70	*T. mentagrophytes*	*T. tonsurans*	*T. tonsurans*	*T. tonsurans*	*T. tonsurans*
74	*T. rubrum*	*T. tonsurans*	*T. tonsurans*	*T. tonsurans*	*T. tonsurans*
67	*T. rubrum*	*T. mentagrophytes*	*T. mentagrophytes*	*T. mentagrophytes*	*T. mentagrophytes*
80	*T. tonsurans*	*T. rubrum*	*T. rubrum*	*T. rubrum*	*T. rubrum*
84	*M. canis*	Nonsporulating mold	*T. rubrum*	*T. rubrum*	*T. rubrum*
89[a]	*M. canis*	*M. canis*	No ID	*M. canis*	*M. canis*
90[a]	*M. canis*	*M. canis*	No ID	*M. canis*	*M. canis*

[a] Samples 89 and 90 are different colony types from the same culture.
[b] No ID, isolate could not be identified.

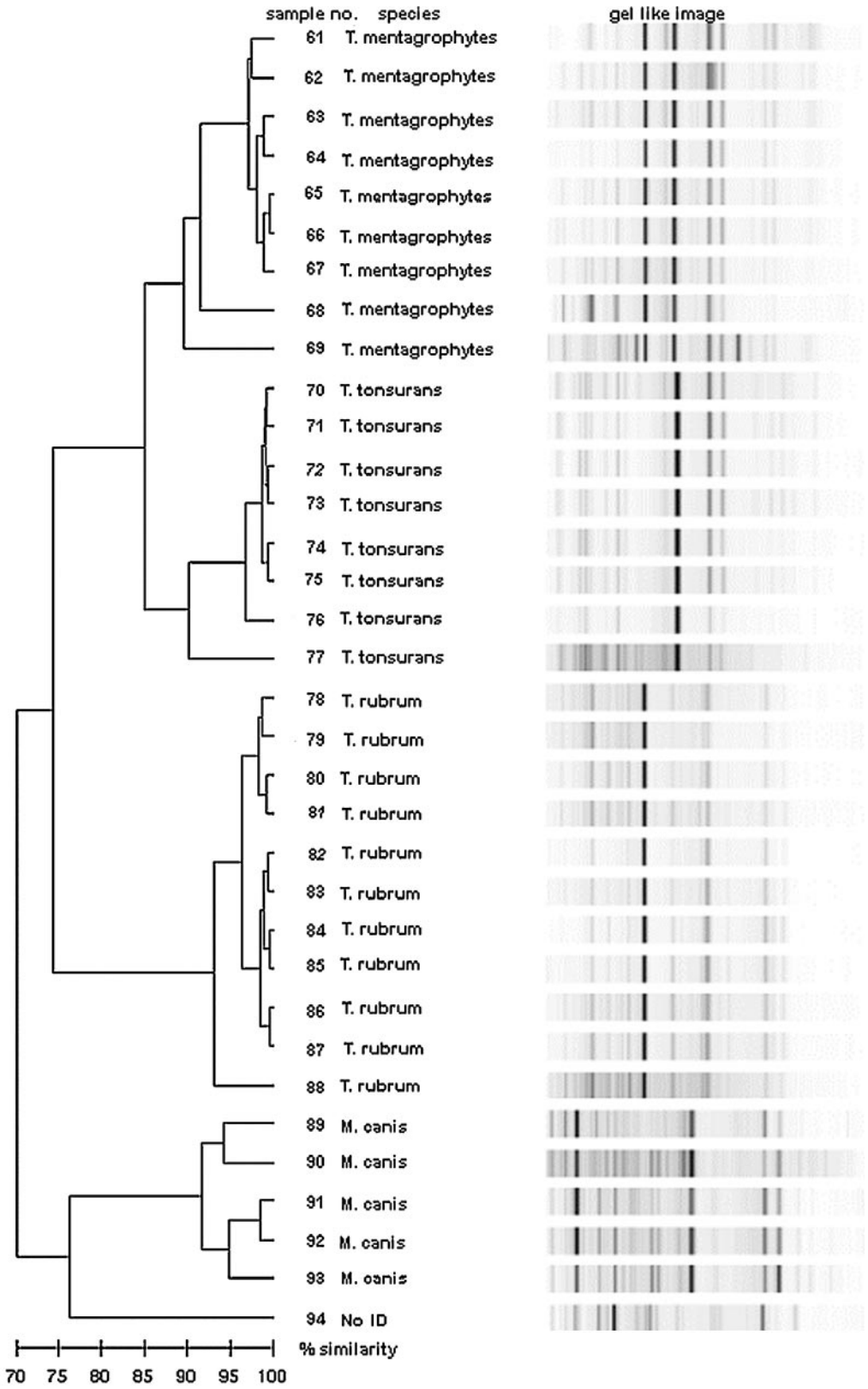

FIG. 2. Dendrogram of dermatophyte cultures tested at ARUP in the second portion of Phase 2 of the study, excluding the contaminated culture. The horizontal bar at the bottom left of the dendrogram indicates the percent similarity coefficient within the species. Sample numbers 89 and 90 are different colony types from the same culture. No ID, isolate could not be identified.

gypseum samples (Fig. 1, nos. 59 and 60), and therefore was not identified. Failure to identify this culture may be due to the small number of *M. gypseum* cultures in the database, and it is conceivable that the addition of more *M. gypseum* cultures would resolve this problem. The unique fingerprint patterns of the single cultures of *E. floccosum* (Fig. 1, no. 57) and *M. nanum* (Fig. 1, no. 19) are considered accurate because they had less than 85% similarity to other fungi in the database, but this should be confirmed by testing additional cultures of these species.

Analysis of the phase 2 data confirmed that rep-PCR results were accurate and reproducible. After initial testing of the cultures in phase 2 (excluding the culture that was inadvertently discarded before repeat testing could be performed and the one that was overgrown with bacteria), agreement between rep-PCR and conventional testing was 88.7% (55 of 62). After discrepancies were resolved, agreement was 100%, based on repeating the conventional tests, rep-PCR, and ITS sequencing. With the exception of the culture that contained two colony variants of *M. canis* (Table 2 and Fig. 2, nos. 89 and 90), which could not be identified when first tested by rep-PCR, the initial rep-PCR results were correct. The unique rep-PCR fingerprint pattern of the nonsporulating mold (by conventional tests) that was identified as *M. audouinii* (Table 2, no. 94) by ITS sequencing is considered correct because *M. audouinii* was not in the database, and the fingerprint pattern of this culture had less than 85% similarity to other fungi in the database. However, as with the single cultures of *E. floccosum* (Fig. 1, no. 57) and *M. nanum* (Fig. 1, no. 19), this should be confirmed by testing additional cultures of this species. Additionally, the specificity of the system should be further evaluated by testing fungi that could resemble dermatophytes early in their growth, i.e., white, fluffy molds, such as *Chrysosporium* and *Emmonsia* spp.

In addition to accuracy, the technical aspects of the DiversiLab system must be considered. In our opinion, several factors make this system appealing for a high-volume clinical laboratory. It can be used not only to identify dermatophytes but also for identification of *Aspergillus* spp. (7), *Candida* spp. (2), and, potentially, mycobacteria (H. Neal, G. Neerings, C. Shutt, and G. Woods, Abstr. 104th Gen. Meet. Am. Soc. Microbiol. 2004, abstr. C-208, p. 160-161, 2004) and for strain typing of many bacteria (9) and certain fungi. More than one type of test (e.g., dermatophyte identification and *Staphylococcus aureus* strain typing) can be done with the same chip. The DiversiLab system uses standardized reagents that are commercially available in kit form, and the web-based software generates user-friendly reports that can be customized for the testing laboratory. The turnaround time for both identification and typing results is <24 h once a pure culture is available for the DiversiLab system versus several days to weeks for fungal identification and 2 to 3 days when using sequencing for identification or pulsed-field gel electrophoresis for bacterial strain typing.

When using the DiversiLab system for dermatophyte identification, all work should be done in a biosafety level 2 biological safety cabinet until cells are lysed, because young dermatophyte cultures (i.e., white, fluffy molds) may resemble young cultures of dimorphic fungi. In general, use of the DiversiLab system for dermatophyte identification does not require extensive experience in mycology. A possible protocol for its use is as follows. When a culture of skin, hair, or nails is positive for a white, fluffy mold on a medium containing cycloheximide, mycelial growth is transferred to a PDA slant (or other similar medium) to obtain a pure culture. When growth on the PDA slant reaches approximately 2 square centimeters, rep-PCR is performed. If rep-PCR fails to provide an identification, the culture is critically examined to ensure purity, and a slide culture is prepared. Testing a pure culture is important, because fungal cultures contaminated with another fungus or with bacteria do not cluster with known fungi in the database, and no identification is provided. However, failure of the DiversiLab system to identify an isolate should trigger further investigation; there may be not only contamination of the culture but also more than one colony morphology of the same species present, or the species may not be in the database.

There are potential limitations to the DiversiLab system. The currently recommended DNA extraction procedure, although technically simple, is very labor-intensive. In our opinion, this will be a major issue for laboratories considering implementation of the system. Although the reagent kits include positive and negative controls, there is no comparison library for the positive control. It is critical that the sample tested be a pure culture. Occasionally, bubbles form when loading the wells of the microfluidic chip. When this happens, the amplified product must be retested, which increases both the turnaround time and cost of the assay. Additionally, the occurrence of electrical interference when the microfluidic chip is being analyzed within the Agilent 2100 bioanalyzer, the presence of dust in the bioanalyzer, or excess vibration can produce an electrical spike in the electropherogram, necessitating repeat testing of the amplified DNA. Finally, for the DiversiLab system to be most cost-efficient, 12 samples plus a positive control (13 samples if no control is run) must be tested, because all 13 wells of the microfluidic chip must contain DNA marker and the gel-dye matrix, even if no sample is being tested.

In summary, the results of our evaluation showed that the performance of the DiversiLab system for identification of the dermatophytes commonly encountered in a clinical mycology laboratory—*T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, and *M. canis*—was excellent. Moreover, the DiversiLab system is technically simple and provides results in <24 h, which is considerably more rapid than conventional identification tests. The DiversiLab system will become a more powerful identification tool as the database is expanded by the addition of more dermatophyte species and other fungi, especially molds with similar-appearing colony morphologies.

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REFERENCES

1. **Bistis, G. N.** 1959. Pleomorphisms in the dermatophytes. Mycologia **51:**440–452.
2. **Chau, A. S., C. A. Mendrick, F. J. Sabatelli, D. Loebenberg, and P. M. McNicholas.** 2004. Application of real-time quantitative PCR to molecular analysis of *Candida albicans* strains exhibiting reduced susceptibility to azoles. Antimicrob. Agents Chemother. **48:**2124–2131.
3. **Graser, Y., A. F. A. Kuijpers, M. El Fari, W. Presber, and G. S. De Hoog.** 2000. Molecular and conventional taxonomy of the *Microsporum canis* complex. Med. Mycol. **38:**143–153.

4. **Graser, Y., M. El Fari, R. Vilgalys, A. F. A. Kuijpers, G. S. De Hoog, W. Presber, and H. J. Tietz.** 1999. Phylogeny and taxonomy of the family *Arthrodermataceae* (dermatophytes) using sequence analysis of the ITS region. Med. Mycol. **37:**105–114.
5. **Graser, Y., A. F. A. Kuijpers, W. Presber, and G. S. De Hoog.** 1999. Molecular taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*. Med. Mycol. **37:**315–320.
6. **Graser, Y., A. F. A. Kuijpers, W. Presber, and G. S. De Hoog.** 2000. Molecular taxonomy of the *Trichophyton rubrum* complex. J. Clin. Microbiol. **38**: 3329–3336.
7. **Healy, M., K. Reece, D. Walton, J. Huong, K. Shah, and D. P. Kontoyiannis.** 2004. Species identification and strain differentiation of *Aspergillus* clinical isolates using automated rep-PCR. J. Clin. Microbiol. **42:**4016–4024.
8. **Larone, D. H.** 2002. Medically important fungi: a guide to identification, 4th ed. ASM Press, Washington, D.C.
9. **Shutt, C. K., J. I. Pounder, S. R. Page, B. J. Schaecher, and G. L. Woods.** 2005. Clinical evaluation of the DiversiLab microbial typing system using repetitive-sequence-based PCR for characterization of *Staphylococcus aureus* strains. J. Clin. Microbiol. **43:**1187–1192.
10. **Sutton, D. A., A. W. Fothergill, and M. G. Rinaldi.** 1998. Guide to clinically significant fungi. Williams and Wilkins, Baltimore, Md.
11. **White, T. J., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Snisky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., San Diego, Calif.