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Evaluation of New Medium for Identification of Dermatophytes and Primary Dimorphic Pathogens

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Only 25 of 77 dermatophytic isolates caused dermatophyte identification medium (DIM) to turn purple after incubation at the recommended temperature (37°C); the accuracy of the results was improved at 30°C (71 of 77 isolates yielded positive results). Many dimorphic pathogenic fungi also tested positive at both incubation temperatures. Thus, DIM has limited usefulness for presumptive identification of dermatophytes.

Dermatophyte test medium (DTM; commercially available from Remel Inc., Lenexa, Kans., and Difco, Detroit, Mich.) was developed during the Vietnam War for rapid and presumptive identification of dermatophytes from soldiers with ringworm infections (1, 11, 12). Observation of color change during culture from clinical specimens (nail clippings, skin scrapings, hair, etc.) placed directly on DTM provided presumptive identification of dermatophytes (2, 3, 10). However, it was reported that nondermatophytic fungi also caused a color change on DTM, thereby giving false-positive results (9). Since many of these nondermatophytic fungi have colony morphologies similar to those of dermatophytes or are routinely isolated from clinical specimens, a positive result with DTM may lead to misidentification of these organisms as dermatophytes (5). A new medium, termed dermatophyte identification medium (DIM), was developed to overcome the reported limitations of DTM (9). Improvements introduced in DIM included incubation at 37°C and the use of an increased concentration of cycloheximide (9). In the present study, we evaluated DIM with various dermatophytes as well as with three genera of dimorphic pathogenic fungi because these two groups of organisms have similar colony morphologies and are sometimes isolated from similar clinical specimens.

Test organisms. Clinical isolates submitted for routine identification to the Mycology Laboratory, Wadsworth Center, were used in the study. A total of 117 isolates, including 77 dermatophytes and 40 primary dimorphic pathogens, were studied. Dematiaceous molds, zygomycetes like *Rhizopus* species and *Mucor* species, various species of *Aspergillus*, common laboratory contaminants (saprobic fungi), and pathogenic yeasts were not included, since they can be initially differentiated based on colony morphology.

DIM preparation. DIM containing dextrose (20 mg/ml), neopeptone (10 mg/ml), cycloheximide (4 mg/ml), penicillin (20 U/ml), streptomycin (40 U/ml) and bromocresol purple (1.6 mg/ml) was prepared as described previously (9). Dextrose, neopeptone, and agar were dissolved in water. Bromocresol purple solution was prepared and added to the above solution, which was then autoclaved. The medium was cooled and aseptically filter sterilized. Then, penicillin, streptomycin, and cycloheximide were added, the pH level was adjusted to within a range of 5.5 to 5.7, and the medium was dispensed into tubes. The medium is light green, and it can be stored at 4 to 8°C for 4 to 6 months.

DIM studies. A portion of each original culture was grown for 5 to 7 days on Sabouraud dextrose agar (Difco) containing 40 μg of gentamicin per ml and 25 μg of chloramphenicol per ml (herein, the supplemented agar is referred to as SAB+ medium). The cultures were aseptically transferred onto two slants containing SAB+ medium and two slants containing DIM, and these slants were incubated at 30 and 37°C for 5 to 7 days. These slants were observed daily for growth on SAB+ medium and for color change from light green to purple on DIM. The results were considered to be negative if either no growth was seen on SAB+ medium or no purple color was evident on DIM. *Trichophyton mentagrophytes* (strain NYS1356-84; Mycology Laboratory, Wadsworth Center) was used as positive control, and uninoculated medium slants served as negative controls. All tests were repeated twice.

The results are summarized in Tables 1 and 2. Among the dermatophytes grown on DIM, 71 isolates cultured at 30°C and 25 isolates cultured at 37°C changed color from light green to purple (Table 1). Among the commonly isolated dermatophytes grown on DIM, 17 out of 21 *Trichophyton tonsurans* isolates and 12 out of 16 *Trichophyton rubrum* isolates did not change color at 37°C (Table 1). Among the dimorphic pathogens grown on DIM, 30 isolates cultured at 30°C and 21 isolates cultured at 37°C changed color (Table 2). It was interesting that 21 isolates of *Coccidioides immitis* yielded positive results while no *Blastomyces dermatitidis* or *Histoplasma capsulatum* isolates grown on DIM changed color at 37°C.

T. tonsurans is the most common pathogen causing tinea capitis in the United States (6). It is most frequently isolated from the hair and scalp in clinical laboratories and physicians' offices. Even though the fungus grew well at 37°C on SAB+ medium, it gave false-negative results on DIM. Similarly *T. rubrum* is also a commonly encountered dermatophyte causing tinea pedis. It grew at 37°C on SAB+ medium but did not change color on DIM. It is relevant to recall that the concentration of cycloheximide in DIM (4 mg/ml) is eight times

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TABLE 1. Comparison of growth of dermatophytes on SAB+ medium and DIM

Organism	No. of isolates positive/no. tested			
	Growth on SAB+ medium		Color change to purple on DIM	
	30°C	37°C	30°C	37°C
Epidermophyton floccosum	1/1	1/1	0/1	0/1
Microsporum canis	6/6	6/6	6/6	0/6
Microsporum cookei	1/1	1/1	1/1	1/1
Microsporum fulvum	2/2	2/2	2/2	2/2
Microsporum nanum	3/3	3/3	3/3	0/3
Microsporum praecox	3/3	1/3	3/3	0/3
Microsporum persicolor	1/1	1/1	1/1	0/1
Trichophyton megninii	1/1	1/1	1/1	1/1
Trichophyton mentagrophytes	6/6	6/6	6/6	5/6
Trichophyton rubrum	16/16	16/16	16/16	4/16
Trichophyton schoenleinii	2/2	1/2	0/2	0/2
Trichophyton simii	3/3	3/3	3/3	3/3
Trichophyton soudanense	2/2	2/2	1/2	0/2
Trichophyton terrestre	2/2	0/2	2/2	0/2
Trichophyton tonsurans	21/21	20/21	21/21	4/21
Trichophyton verrucosum	5/5	5/5	5/5	5/5
Trichophyton violaceum	2/2	0/2	0/2	0/2
Total	77/77	69/77	71/77	25/77

higher than that in DTM (0.5 mg/ml). A previous report documented that a higher concentration of cycloheximide (5 mg/ml) inhibited the growth of *T. rubrum* and *H. capsulatum*, while these organisms grew well at a lower concentration (8). It was also reported that the organisms' responses to cycloheximide were variable and depended upon various factors like the concentration of cycloheximide, the duration of exposure of the organism to the drug, species specificity, and the development of resistance during exposure to cycloheximide (8). Interestingly, cycloheximide was also reported to cause inhibition of the yeast form of *B. dermatitidis* at 37°C without affecting mycelial growth at 30°C (7). Of the other important fungal pathogens, *Cryptococcus neoformans* is also sensitive and does not grow on media containing cycloheximide, even at the lowest concentration (4). It can be reasonably speculated that the high concentration of cycloheximide in DIM inhibited dermatophytic fungi at 37°C. This observation was in contrast with the results of an earlier study, in which the high specificity and sensitivity of DIM were attributed to high concentrations of cycloheximide and incubation of cultures at a high temperature (9). All isolates of *Trichophyton verrucosum* grew well on DIM and caused a change in color at both temperatures. This result was not in agreement with the previous report, where one isolate of *T. verrucosum* did not change the DIM color (9).

TABLE 2. Comparison of growth of dimorphic pathogenic fungi on SAB+ medium and DIM

Organism	No. of isolates positive/no. tested			
	Growth on SAB+ medium		Color change to purple on DIM	
	30°C	37°C	30°C	37°C
Blastomyces dermatitidis	3/3	0/3	2/3	0/3
Coccidioides immitis	29/29	21/29	27/29	21/29
Histoplasma capsulatum	8/8	2/8	1/8	0/8
Total	40/40	23/40	30/40	21/40

It has been previously reported that certain nonpathogenic dermatophytoids like *Trichophyton ajelloi* and arthrodermataceous fungi like *Chrysosporium* and closely related fungi gave positive reactions on DTM and DIM (5, 9, 10). Similarly, we found that an arthrodermataceous fungus, *C. immitis*, changed the DIM color at 37°C and grew well in mold form. These results were in contrast with the results of an earlier study, in which *C. immitis* was reported not to change the medium color and no growth was seen at 37°C (9). *C. immitis* is a highly infectious fungal pathogen that often forms white colonies, resembling the colonies of dermatophytes, and its false positivity on DIM may pose a hazard for laboratory personnel (5). The obvious limitation of our study includes the use of pure cultures instead of a sample matrix that mimics dermatological specimens. It is also not known if saprobic fungi present as contaminants in the clinical specimens would influence the performance of DIM.

In our evaluation, DIM was associated with significantly high false-negative results with common dermatophytes like *T. rubrum* and *T. tonsurans* at 37°C, but the results improved when the incubation temperature was 30°C. For primary dimorphic pathogens, the number of false-positive results was slightly lower when the incubation temperature was increased. Thus, DIM has high false negatives and false positives at its recommended formulations and usage, which limits its usefulness for presumptive identification of dermatophytes.

REFERENCES

1. **Allen, A. M., D. Taplin, J. A. Lowy, and L. Twigg.** 1972. Skin infections in Vietnam. Mil. Med. **137:**295–301.
2. **Carroll, H. F.** 1974. Evaluation of dermatophyte test medium for diagnosis of dermatophytes. J. Am. Vet. Med. Assoc. **165:**192–195.
3. **Elewski, B. E., J. Leyden, M. G. Rinaldi, and E. Atillasoy.** 2002. Office practice-based confirmation of onychomycosis: a US nationwide prospective survey. Arch. Intern. Med. **162:**2133–2138.
4. **Fuentes, C. A., F. Trespalacios, G. F. Baquero, and R. Aboulafia.** 1952. Effect of actidione on mold contaminants and on human pathogens. Mycologia **44:**170–175.
5. **Kane, J., and R. C. Summerbell.** 1999. *Trichophyton*, *Microsporum*, *Epidermophyton*, and agents of superficial mycoses, p. 1275–1298. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. **Lobato, M. N., D. J. Vugia, and I. J. Frieden.** 1997. Tinea capitis in California children: a population-based study of a growing epidemic. Pediatrics **99:**551–554.
7. **McDonough, E. S., L. Ajello, L. K. George, and S. Brinkman.** 1960. In vitro effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab. Clin. Med. **55:**116–119.
8. **Salkin, I. F., and N. Hurd.** 1972. Quantitative evaluation of the antifungal properties of cycloheximide. Antimicrob. Agents Chemother. **1:**177–184.
9. **Salkin, I. F., A. A. Padhye, and M. E. Kemna.** 1997. A new medium for the presumptive identification of dermatophytes. J. Clin. Microbiol. **35:**2660–2662.
10. **Sinski, J. T., J. R. Swanson, and L. M. Kelley.** 1972. Dermatophyte test medium: clinical and quantitative appraisal. J. Investig. Dermatol. **58:**405–411.
11. **Taplin, D., N. Zaias, G. Rebell, and H. Blank.** 1969. Isolation and recognition of dermatophytes on a new medium (DTM). Arch Dermatol. **99:**203–209.
12. **Taplin, D.** 1972. Dermatophyte test medium. J. Ivestig. Dermatol. **58:**412.