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Multicenter Evaluation of Microring YT, a New Method of Yeast Identification

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Microring YT was evaluated and compared with established methods for the identification of 142 clinical yeast isolates. Only 75 isolates (52.8%) were correctly identified by Microring YT. Results with this test were often difficult to read and subject to interlaboratory variations.

The incidence of systemic yeast infections, in particular those caused by *Candida* and *Cryptococcus* species, is increasing (2) and, as many of these infections can be fatal in immunocompromised patients, it is important that the causative organisms are identified quickly. As a result, several new rapid methods of yeast identification have become available commercially (3, 4). It is important, however, that the increased rapidity of these methods should not be achieved at the expense of accuracy.

One new method (Microring YT; Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom) is an extension of the work published by Sobczak (5) and is based on the different susceptibilities of yeasts to six chemicals or dyes, namely, Janus green, ethidium bromide, triphenyl tetrazolium chloride, brilliant green, cycloheximide, and rhodamine 6G. A Microring consists of filter paper disks impregnated with these chemicals, which are evenly distributed around a filter paper ring (Fig. 1).

In this study, the medical mycology laboratories at the Bristol Royal Infirmary (Bristol), the University of Glasgow (Glasgow), and the General Infirmary (Leeds) in the United Kingdom each collected 40 to 50 yeast isolates recovered from clinical specimens during the study period. With the exception of three germ tube-positive isolates, all others included in the study were negative in the germ tube test (6). All yeast isolates were circulated to the three participating laboratories for identification by Microring YT and were tested within 6 weeks of their initial isolation. In addition, the identities of the yeasts were determined by the Wickerham assimilation and fermentation methods (1) and by API 20C AUX (API-Bio Merieux, Basingstoke, United Kingdom). All yeasts were tested in a blind fashion by the three methods, and results were compiled at the end of the study period.

A total of 142 yeast isolates were included in the study. Assimilation and fermentation tests were performed as described elsewhere (1), and API 20C AUX was performed in accordance with the manufacturer's instructions. The API 20C system used in this study included a semisolid basal medium which did not require melting before inoculation. For identification of the yeasts by Microring YT, test isolates were initially cultured on Sabouraud glucose agar (CM41; Oxoid Ltd., Basingstoke, United Kingdom) slants for 24 h at 37°C. A light suspension (equivalent to a McFarland no. 2-3 standard) of each isolate was made in sterile saline and spread over the surface of a Sabouraud's glucose agar plate with a swab. The surface was allowed to dry, and a Microring was placed in the center of the plate and pressed down gently to ensure good contact with the agar surface. Yeast identities were determined by considering a number of different features on the plates after incubation at 37°C for 24 to 48 h. Features aiding identification included the presence and the sizes of the inhibition zones around each disk on the ring and the color of the yeast growth around disk 3 (triphenyl tetrazolium chloride). Regrowth within inhibition zones, a characteristic of some yeast species, was also noted. Any inhibition zone around a disk was regarded as inhibition of growth and received a score which corresponded to the number of the disk; zones with no growth were scored as 0, and zones with regrowth were scored as the number of the disk plus the suffix R. Results were scored from 1 to 6 to yield a six-digit code (Fig. 1). The sizes of the inhibition zones were also measured, and the identities of the yeast isolates were determined by comparing the codes, zone sizes, presence of regrowth, and color around disk 3 with a list of identification codes supplied with the Microrings.

Of the 142 yeast isolates studied, identifications by assimilation and fermentation and by API 20C AUX were in agreement in all cases. Fourteen different yeast species were studied, including representatives from the genera *Candida*, *Torulopsis*, *Cryptococcus*, *Saccharomyces*, *Trichosporon*, and *Blastoschizomyces*. Only three isolates of *Candida albicans* were included in the study, as preliminary work showed that this species was easy to identify with Microring YT (V. Hopwood, L. McHugh, and G. S. Shankland, Abstr. Rev. Iber. Micol., 5:60, 1988).

Table 1 shows the number of each of the 14 yeast species tested and the number of laboratories in which they were correctly identified by Microring YT. Only 75 isolates (52.8%) yielded the same results in all three laboratories and showed agreement between Microring YT and established identification methods. *Candida parapsilosis* was the most difficult species to identify by Microring YT. Seven of the 45 isolates of *C. parapsilosis* were not identified at all in any of the laboratories, and only 12 isolates were identified correctly in all three. Identification of *Candida guilliermondii*

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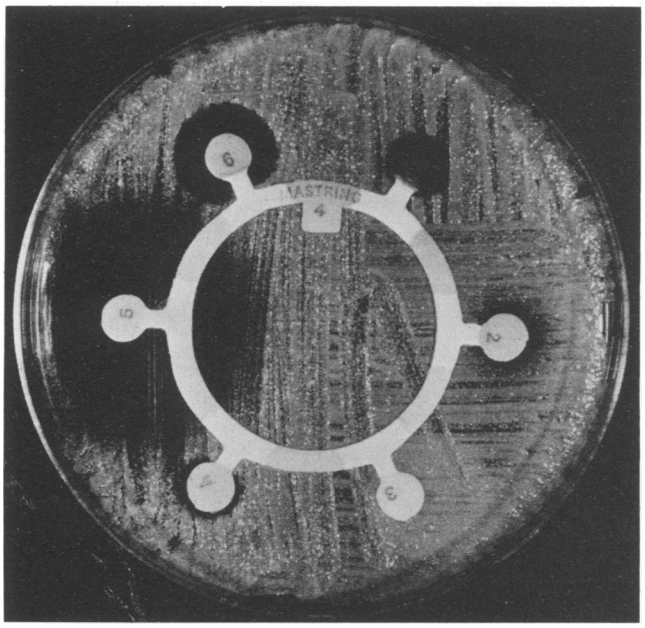

FIG. 1. *Candida lusitaniae* identified by Microring YT. The identification profile based on the inhibition zones is 120456, with a large zone around disk 5.

was also difficult by Microring YT. None of the three isolates of *C. guilliermondii* were correctly identified in all three laboratories.

Interlaboratory variations in yeast identities with Microring YT were apparent, as were variations when the same batch of plates was read by more than one person. Some yeast species were incorrectly identified by Microring YT because the profiles obtained corresponded to those of another yeast species. Other isolates were not identifiable from the Microring YT profiles obtained; some isolates yielded profiles which were not present in the data base, whereas others yielded profiles whose zone sizes did not match those in the data base.

Of the 426 profiles obtained by the three laboratories for the 142 isolates, 299 led to correct identification of the yeast isolates. Correct identities were obtained for 75 isolates (225 profiles) in all three laboratories, 30 isolates (60 profiles) in two laboratories, and 13 isolates (13 profiles) in only one laboratory. Fifty-six further profiles led to incorrect identifications of the yeast isolates with the present data base; 28 profiles did not correspond to any in the data base; and 37 profiles were identical to existing profiles, but their zone sizes did not match (Table 2). In seven instances the yeast isolates failed to grow on the test plates or did not yield a readable profile.

TABLE 1. Number of yeast isolates correctly identified by Microring YT in three laboratories

Yeast species (no. of isolates tested)	No. of isolates correctly identified in:			
	All three laboratories	Two laboratories	One laboratory	No laboratory
Candida albicans (3)	3			
Candida guilliermondii (3)		1	1	1
Candida krusei (12)	11	1		
Candida lusitaniae (9)	7	1		1
Candida parapsilosis (45)	12	16	10	7
Candida pseudotropicalis (8)	7	1		
Candida rugosa (1)			1	
Candida tropicalis (22)	15	6		1
Torulopsis candida (5)	4	1		
Torulopsis glabrata (16)	14	1		1
Saccharomyces cerevisiae (6)	2	2	1	1
Cryptococcus albidus (1)				1
Trichosporon beigelii (7)				7
Blastoschizomyces capitatus (4)				4

TABLE 2. Reasons for yeast species being incorrectly identified or not identified by Microring YT[a]

Yeast species	No. of:		
	Isolates incorrectly identified	Profiles	
		Not in data base	With incorrect zone sizes
Candida guilliermondii	4	1	
Candida krusei	1		
Candida lusitaniae	3		1
Candida parapsilosis	19	16	20
Candida pseudotropicalis		1	
Candida rugosa	2		
Candida tropicalis	9		
Torulopsis candida	1		
Torulopsis glabrata	2		2
Saccharomyces cerevisiae	4	2	1
Cryptococcus albidus			3
Trichosporon beigelii	7	2	8
Blastoschizomyces capitatus	4	6	2

[a] Yeast isolates which failed to grow on the test plates were not included.

Of the yeast species which were incorrectly identified, the most common confusions existed between *C. guilliermondii* and *Torulopsis candida*, *Torulopsis glabrata*, and *Saccharomyces cerevisiae* and between *Candida tropicalis* and *Candida pseudotropicalis*. The data base used in this study did not contain profiles for the genus *Trichosporon*. The *Trichosporon beigelii* and *Blastoschizomyces capitatus* isolates which were incorrectly identified yielded profiles corresponding to those for *C. albicans*. These were the only isolates which were falsely identified as *C. albicans*. There was no particular pattern for the other species which were incorrectly identified.

Although the Microring YT for yeast identification is simple to use and relatively inexpensive, this preliminary study suggests that accurate identification of medically important yeast species by this method is difficult. Interpretation of the plates is often subject to reader variations, and in some cases problems exist in determining what constitutes an inhibition zone. Most isolates which were difficult to identify by Microring YT were easy to identify by established procedures of assimilation and fermentation and by API 20C AUX. The usefulness of the test is limited by the small number of yeast species included in the data base. The data base used in this study included profiles for only 18 yeast species. Although the data base can be expanded, it seems unlikely that the other problem, namely, reproducibility among laboratories and individuals, can be overcome satisfactorily.

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