

# Evaluation of the Microring YT system for identifying clinical yeast isolates

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## Abstract

The Microring YT is a commercial system for identifying clinical yeast isolates. The system uses a series of discs impregnated with inhibitory agents mounted on a ring. The pattern of growth and inhibition produced provides a six digit code which can be compared with a table provided by the manufacturer. The performance of this system was compared with the API 32C in the identification of 606 yeast isolates (355 clinical and 251 environmental strains). The Microring YT system was in 72.6% agreement with the API 32C system. The sensitivity of identification of different species varied from 38% to 100%.

The API 32C system has a more extensive database than the Microring YT and is thus more reliable for use, but it is considerably more expensive. It is concluded that although the Microring YT is cheap, easy, and convenient to use, it is inadequate for many common *Candida* species.

The prevalence of opportunistic yeast infections is increasing, especially in renal transplant recipients, oncology and other immunocompromised patients.<sup>1,2</sup> Many of these infections are fatal so rapid identification of an infecting strain is important, especially as some strains show natural or acquired antifungal resistance—for example, *Candida lusitanae*.<sup>2</sup> Accurate identification permits a better understanding of the clinical importance of various yeast species and allows mycoserological tests to be interpreted. Conventional identification methods are time consuming and complex. Consequently several new methods of yeast identification have become available for clinical use, most offering convenience in addition to rapidity.<sup>3,4</sup>

The Microring YT is a new commercial system based on a test devised by Sobczak.<sup>5</sup> It uses the differing susceptibilities of yeast strains to six discs (mounted on a filter paper ring) which are impregnated with chemicals or dyes (Janus green, ethidium bromide, triphenyl tetrazolium chloride, brilliant green, cycloheximide and rhodamine 6G) to generate a six digit code. The code is compared with a list of profiles provided by the manufacturer.

We compared the Microring YT to an established system (API 32C) to determine whether it was a rapid, reliable, and accurate method that could be used easily in a diagnostic microbiology laboratory.

## Methods

A total of 606 yeast strains were examined, representing 13 yeast species. Three hundred and fifty five (13 species) were recent clinical isolates from patients on the intensive care unit and 251 (nine species) were environmental isolates collected from the unit over the same seven month period. Clinical specimens were collected from multiple sites from each patient—for example, throat, rectal, urine, wound, interdental. Thirty eight (9%) clinical isolates were repeat isolates from the same site but after an interval of at least seven days.

All strains were germ tube negative. Germ tube positive isolates were not included as these would normally be identified as *Candida albicans* and no further identification undertaken.<sup>6</sup> All isolates were stored on Sabouraud's glucose agar (SGA) slopes (CM 41; Oxoid Ltd, Basingstoke, United Kingdom) at room temperature and tested within six weeks of isolation. All strains were subcultured on to SGA plates to check for purity before testing. Two commercial kits were used to identify the organisms, the API 32C (API-BioMérieux, Basingstoke, United Kingdom) and the Microring YT (Medical Wire and Equipment Co Ltd, Corsham, United Kingdom).

The API 32C was performed according to the manufacturer's instructions. Kits were stored at 4°C until used. After incubation at 30°C for 48 hours the biotype number of the unknown yeast was compared with those in a database compendium supplied by the manufacturer.

With the Microring YT system, the test organism was emulsified in 3 ml sterile distilled water to give a density equivalent to a No 1–No 2 McFarland standard. This was spread across the surface of an SGA plate with a sterile swab. When the surface had dried, a Microring was placed in the centre of the plate with sterile forceps and gently pressed down to ensure good contact with the surface of the agar. After incubation at 37°C for 24 to 48 hours the plates were examined for zones of growth inhibition, the colour of the yeast growth, and the presence of regrowth within any inhibition zone around the discs. Each disc on the Microring is numbered 1–6. Inhibition of growth around a disc was scored with the number of that disc; absence of inhibition was scored as 0. Thus a six digit code was generated—for example, 120406. This code was compared with a list of profile numbers and zone sites provided by the manufacturer. Inhibition zone sizes, presence of regrowth, and coloration of the growth were used to discriminate between species giving the same code. If the code given by the unknown

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Table 1 Comparison of Microring YT and API 32C systems for identification of clinical and environmental isolates

API 32C	Microring YT	
	Clinical and environmental isolates	
	Agreement	"Incorrect" identification*
<i>Candida albicans</i>	158/166	<i>Candida pseudotropicalis</i> (5) No identity (2) <i>Candida parapsilosis</i>
<i>Candida krusei</i>	4/4	No identity (5)
<i>Candida glabrata</i>	58/65	<i>Candida krusei</i> (2) <i>Candida albicans</i> (4) <i>Candida krusei</i> (3)
<i>Candida lusitanae</i>	44/51	<i>Candida lusitanae</i> (46) No identity (26) <i>Candida albicans</i> (14) <i>Geotrichum</i> sp (14) <i>Candida krusei</i> (11) <i>Candida glabrata</i> (4) <i>Candida tropicalis</i>
<i>Candida parapsilosis</i>	100/220	<i>Saccharomyces cerevisiae</i> <i>Candida guilliermondii</i> <i>Candida famata</i> <i>Candida pseudotropicalis</i> <i>Candida famata</i> (9) <i>Candida lusitanae</i> (4) <i>Candida krusei</i> (2)
<i>Saccharomyces cerevisiae</i>	3/7	No identity (2) <i>Candida glabrata</i> (2)
<i>Candida tropicalis</i>	53/55	<i>Candida albicans</i> <i>Candida pseudotropicalis</i>
<i>Candida famata</i>	2/2	

\*For the purpose of comparison, API 32C was assumed to be "correct".

yeast was not in the list of profile numbers or if the zone sizes, regrowth, or coloration were discrepant, the isolate was recorded as "no identity". Isolates were processed blind and at random and all plates were read by the same operator.

In this study it was assumed that the identities given by the API 32C were correct, and the performance of the Microring YT was assessed against this standard.

The sensitivity and specificity of detection and the predictive value of a positive and a negative test were determined for each species identified by the Microring YT.<sup>7</sup>

## Results

With the API system, 13 different species were identified among the patient isolates and nine of these were found in the environment (table 1). Only one species (*Candida famata*) was found exclusively in the environment so patient and environmental isolates were combined. One hundred and sixty six of the 606 germ tube negative isolates under investigation were *C. albicans*. This represents 36% of all *C. albicans*

Table 2 Sensitivity and specificity of species identification with Microring YT, with predictive values of identification

	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
<i>Candida albicans</i>	95	98	95	98
<i>Candida krusei</i>	100	97	22	100
<i>Candida glabrata</i>	89	99	91	99
<i>Candida lusitanae</i>	86	91	47	99
<i>Candida parapsilosis</i>	45	100	100	76
<i>Saccharomyces cerevisiae</i>	43	100	75	99
<i>Candida tropicalis</i>	100	100	98	100
<i>Candida famata</i>	100	98	20	100
<i>Candida guilliermondii</i>	38	100	90	97

Table 3 Identification of species not included in Microring YT database

API 32C	Microring YT	
	Clinical and environmental isolates	
	Agreement	"Incorrect" identification*
<i>Candida melibiosica</i>	0/2	<i>Candida lusitanae</i> (2)
<i>Candida inconspicua</i>	0/6	<i>Candida krusei</i> (4) No identity (2)
<i>Trichosporon capitatum</i>	0/2	No identity
<i>Trichosporon cutaneum</i>	0/2	<i>Candida pseudotropicalis</i> <i>Candida krusei</i> No identity

\*For the purpose of comparison, API 32C was assumed to be "correct".

isolates from the study period. This proportion of germ tube negative strains is higher than might be expected, as 95–97% of clinical isolates of *C. albicans* are germ tube positive in serum.<sup>6</sup> This finding may result from the inclusion of more than one clinical isolate of *C. albicans* from each patient (mean 2.6, range 1–11), especially if patients were extensively colonised with atypical strains.

Nine of the 13 species identified by API 32C were included in the Microring YT database (594 of 606 isolates (98%)).

Four hundred and thirty one of the 594 isolates that were in the Microring YT database were correctly identified (72.6%) (table 1). Thirty five (5.9%) strains could not be identified. Agreement between the API and Microring systems varied between 38 and 100% for different species; it was less than 50% for three species (*Candida parapsilosis*, *Saccharomyces cerevisiae*, and *C. guilliermondii*) (table 1). Notably, *Candida parapsilosis* gave a wide range of misidentifications but was frequently identified as *Candida lusitanae* (20.9%). The predictive value of identification of some species was very poor (table 2), with three species (*Candida krusei*, *C. lusitanae*, and *C. famata*) having values of less than 50%.

Twelve isolates belonged to species not included in the Microring YT database. Only four of these gave "no identity", eight giving "false" identification (table 3).

## Discussion

There has been one previously published evaluation of the Microring YT, where 142 isolates were examined in three laboratories.<sup>8</sup> Only 52.8% of isolates were correctly identified in all laboratories. Our finding that 72.6% of strains were correctly identified may reflect the large numbers of commonly isolated species included in our group of isolates. We found that accurate identification of many species was a problem with the Microring YT. The method sheet supplied with the rings states that any zones, however small, should be scored as susceptible. Although a single observer read all the plates, considerable difficulty was often found in deciding whether there was total inhibition of growth around a disc. Even with experience, interpretation was sometimes very subjective. This would obviously impair reproducibility in most laboratories, where it is unlikely that the same person would read all

tests. Lack of reproducibility may have contributed to the lower identification rate found in the previous study.<sup>8</sup>

The Microring YT has a limited database of only 18 species. This is smaller than other yeast identification systems<sup>3,4</sup> and may be difficult to enlarge with only six tests on each ring and a limited number of discriminatory factors. The sensitivity of detection of several common species was found to be very poor with a positive predictive value of below 50%.

Compared with the API 32C, the Microring YT is very quick and simple to use, requires no special equipment, needs very little storage space and is considerably cheaper (about £0.40/test compared with £2.00/test). This is at the expense of being difficult to read, however, having a limited database and poor sensitivity for several species.

In a routine diagnostic microbiology laboratory most yeast isolates do not require full identification. In the few cases where it is important to identify accurately clinically

important isolates, we feel that the performance of the Microring YT is inadequate, its advantages are outweighed by its disadvantages, and another identification method should be used.

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