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 cultured 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 Micro-rings.

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* Corresponding author.
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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 426 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.

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 beigelli* 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.

We thank Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom, for providing the Microrings and API 20C auxanograms.

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

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>No. of isolates correctly identified in:</th>
<th>All three laboratories</th>
<th>Two laboratories</th>
<th>One laboratory</th>
<th>No laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em> (3)</td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Candida guilliermondii</em> (3)</td>
<td></td>
<td>11</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida krusei</em> (12)</td>
<td></td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida lusitaniae</em> (9)</td>
<td></td>
<td>12</td>
<td>16</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em> (45)</td>
<td></td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida pseudotropicalis</em> (8)</td>
<td></td>
<td>2</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida rugosa</em> (1)</td>
<td></td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida tropicalis</em> (22)</td>
<td></td>
<td>14</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Torulopsis candida</em> (5)</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Torulopsis glabrata</em> (16)</td>
<td></td>
<td>1</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> (6)</td>
<td></td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Cryptococcus albidos</em> (1)</td>
<td></td>
<td>1</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Trichosporon beigelli</em> (7)</td>
<td></td>
<td>7</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><em>Blastoschizomyces capitatus</em> (4)</td>
<td></td>
<td>4</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2. Reasons for yeast species being incorrectly identified or not identified by Microring YT

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>No. of isolates incorrectly identified</th>
<th>No. in data base</th>
<th>With incorrect zone sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida guilliermondii</em></td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida lusitaniae</em></td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>19</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><em>Candida pseudotropicalis</em></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida rugosa</em></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Torulopsis candida</em></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Torulopsis glabrata</em></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Cryptococcus albidos</em></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichosporon beigelli</em></td>
<td>7</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td><em>Blastoschizomyces capitatus</em></td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

* Yeast isolates which failed to grow on the test plates were not included.
LITERATURE CITED

Letters to the Editor
Pathogenicity of Blastocystis hominis

We noted with interest the letters by Rosenblatt (4) and Zierdt (6) concerning the possible pathogenicity of Blastocystis hominis. This discussion is particularly exciting to the microscopist, as B. hominis is probably the second most frequently identified organism (yeasts being the first) in the gut flora. We are concerned that all laboratories are not dealing with the same set of “facts” concerning B. hominis since reports on its prevalence have varied from 0 (5) to 3.2 (2) to 17.5 (3) %.

When B. hominis was first defined (as an artifact or yeast), the abilities of laboratorians to recognize it varied highly. B. hominis was frequently included in the artifact section of atlastes (1), was never included on proficiency tests, and was hardly ever reported. In recent years it has been included, initially as an optional and then as a required organism, in CAP and other state and national survey samples. The recent review by Zierdt (7), however, may be the first publication of extensive high-quality photographs of the organism in its various forms and stages. In our experience it is difficult for the untrained microscopist to identify B. hominis with the simple hematology microscope frequently employed for parasite examinations. The frequency of identification improves dramatically when a microscope with high quality optics is employed. Since the organisms lack a cell wall and the cytoplasm is frequently condensed around the periphery, we employ phase-contrast optics as part of every examination. Additionally, we examine all specimens with a trichrome procedure and have found this stain to be excellent for recognition of B. hominis.

Using these procedures we have identified B. hominis with great frequency. At Meadowlands Clinical Laboratory (Rutherford, N.J.), we found an almost 20% positive rate. At Great Smokies Diagnostic Laboratory we found a 15 to 20% positive rate. As both labs are reference laboratories, receiving most of their specimens from patients visiting physician offices, the prevailing complaints are more chronic than acute. In a separate study of patients with acute gastrointestinal complaints from a largely immigrant population (62% Latin American and 23% Asian) visiting the outpatient GI Clinic of Elmhurst Hospital (Bronx, N.Y.), we observed a positive rate of Blastocystis identification of 60% (42 of 70 patients). Trichrome smears were reread at the Centers for Disease Control in Atlanta, Georgia (100% agreement), confirming the accuracy of our observations. It appears to us that this organism is very prevalent in stool samples from both acutely and chronically ill patients and that a need for improved training programs probably exists. We believe that independent of the status of this organism’s pathogenicity, its presence should always be reported. Only then will physicians and researchers have the data on which to draw conclusions concerning the organism’s medical significance.

As for the question of pathogenicity, we believe that a certain confusion exists with respect to the possible involvement of this organism in chronic compared with acute illnesses. In the case of acute illness, it is important to be able to identify a unique cause and to be able to direct therapy against this cause. The case for pathogenicity of B. hominis in acute illness, although mostly based upon epidemiological evidence, is fairly strong but not conclusive. Clearly, the dialogue and debate are not over. The case for pathogenicity of B. hominis in chronic illness, however, is more complex. We frequently observe B. hominis in patients with diminished levels of Escherichia coli and/or Lactobacillus spp., with high fecal pH values, with low butyrate values, and/or with an overgrowth of Candida spp. These patients often have prolonged transit times and have assorted gastrointestinal complaints, together with a myriad of other complicating symptoms. We suspect that in these patients B. hominis may have a real but weak pathogenicity, contributing to illness as part of a larger picture, including nutritional and digestive components.

REFERENCES

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Asheville, North Carolina 28806

Ed. Note: Dr. Zierdt felt that no response was necessary.

Medical Wire and Equipment Company Microring YT

A study by Shankland et al. (1) was based on a product that was manufactured by Mast Laboratories, Liverpool, United Kingdom, not by Medical Wire and Equipment Company (MW&E). MW&E had contracted Mast Laboratories in 1987 to make the Microring YT. Because of the poor performance of the Mast-manufactured product, which was the product used in the above-referenced article, MW&E severed its manufacturing agreement with Mast Laboratories in 1988. MW&E immediately proceeded to research, develop, and manufacture this product in-house. In May 1990, at the American Society for Microbiology Annual Meeting in Anaheim, Calif., a poster session was presented
by Dr. J. Perry and Dr. G. Miller, showing very favorable results. With a sample size of 572 clinical isolates, the MW&E-manufactured Microring YT correctly identified 93% of the yeast isolates. This led to MW&E receiving Food and Drug Administration (FDA) approval in August 1990. It should also be noted that the data in the article by Shankland et al. are in excess of 3 years old, having been done in 1987 and 1988. Furthermore, the article was submitted and accepted for publication at the same time that MW&E received FDA approval on the product.

We have spoken to Dr. Shankland, one of the article’s authors, who informed us that an addendum was to be published stating exactly what we have said above. Because of what we hope was simply an oversight on the author’s part, the addendum did not accompany the article.

It is evident that this publication has unfairly affected the acceptance of the Microring YT as a useful tool in the identification of yeasts. MW&E is constantly defending our product with the poster session results from the 1990 Annual Meeting, but because of the clout that an article published in the *Journal of Clinical Microbiology* carries, only the acknowledgment by the authors of the article and by the *Journal of Clinical Microbiology* that the Microring YT was unfairly and mistakenly reported to be a poorly performing product will rectify this situation. A clear distinction between the FDA-approved Microring YT and the inferior Mast ring, not approved by the FDA, needs to be understood by all. The ring pictured and used in the study by Shankland et al. is circular, as were the rings manufactured by Mast Laboratories. MW&E’s manufacturing process used only a hexagonal ring, which was the ring receiving FDA approval in August 1990.

We wish to publish this statement in order to rectify this situation once and for all.

Thank you for allowing us this space to voice our concerns and clear up this situation.

REFERENCE


Michael O. Frick

*Medical Wire and Equipment Company*
7 The Boardwalk
Sparta, New Jersey 07871

Author’s Reply

The work published in the *Journal of Clinical Microbiology* was carried out with the full knowledge of Medical Wire & Equipment Co., who supplied us with the materials tested in our study. We have no way of being sure who manufactured the Microrings tested in our study, but the fact that they were supplied to us after Medical Wire & Equipment Co. severed its manufacturing agreement with Mast Laboratories suggests that they may well have been “manufactured in-house” by Medical & Wire Equipment Co. We do not accept that the “Microring YT was unfairly and mistakenly reported” by us for the following reasons.

(i) Medical Wire & Equipment Co. is incorrect to claim that our work was carried out in 1987 and 1988. We note that Medical Wire & Equipment Co. severed its manufacturing agreement with Mast Laboratories in 1988. They “immediately proceeded to research, develop, and manufacture” the product in-house. The work presented in our article was initiated following discussions with Medical Wire & Equipment Co. (UK) in 1988. The Microrings used in our work were supplied subsequent to that discussion.

(ii) It was stated on the product packaging of the Microrings used in our work that they met the MWYT-USA specification. We were supplied with a new data base and product insert at the start of the study. The six dyes and chemicals were of the same concentrations as those used in the currently marketed product.

(iii) N. Sharples, Technical Sales Manager of Medical Wire & Equipment Co. (UK), was kept informed of the progress of the study at the end of 1988 and in 1989. Medical Wire & Equipment Co. agreed to preliminary results being presented at the annual scientific meeting of the British Society for Mycopathology in 1989. There was no indication then that the product they had given us to test was in any way different from that being marketed.

(iv) Medical Wire & Equipment Co. (UK) was sent a copy of our paper in May 1990. They then informed us that other workers (Dr. G. Miller, Dr. J. S. Matthews, and Dr. J. L. Perry) had submitted their work on the product to the *Journal of Clinical Microbiology*. We note this work has not to our knowledge been published in any form other than an abstract.

(v) At no time was it agreed by any of the authors that an addendum to our article should be published.

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