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Comparison of Four Methodologies for Rapid and Cost-Effective Identification of *Candida glabrata*

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To improve turnaround time and decrease the cost of the identification of *Candida glabrata*, we evaluated four methods for the detection of trehalose assimilation or fermentation. These methods were compared with the API 20C method (bioMERIEUX, Hazelwood, Mo.) to determine accuracy. We recommend the use of Remel Rapid Trehalose Assimilation Broth because of its rapid, 3-h results, reasonable sensitivity, and low number of false positives.

Candida glabrata has emerged as an opportunistic pathogen in neonates and an important pathogen in patients with solid tumors as well as nononcologic diseases (3, 5). It ranks fourth among the *Candida* species isolated from blood and has a mortality rate as high as that of *C. albicans* infections (2, 5, 13). A multicenter study showed that *C. glabrata* was responsible for 20% of the *Candida* urinary tract infections (2), and it is also a cause of vaginitis (10, 14). In our setting, *C. glabrata* is the second-most-frequently-isolated yeast from clinical specimens at Associated Regional and University Pathologists (ARUP) Laboratories, Salt Lake City, Utah. Significantly, *C. glabrata* is resistant to many azole antifungal agents, particularly fluconazole (2, 4, 5, 11). Because of its frequency of isolation and decreased sensitivity to the imidazole antifungal agents, a rapid diagnostic test could theoretically impact patient care by affecting therapy selection, especially in cases of candidemia.

Four methods for rapid screening and identification of *C. glabrata* were compared—the Remel Rapid Trehalose Assimilation Broth and the Remel Yeast Fermentation Broth (Remel Laboratories, Lenexa, Kans.), the Trehalose Fermentation Broth (Hardy Diagnostics, Santa Maria, Calif.), and the Mayo Clinic Rapid Assimilation Trehalose Broth described by Stockman and Roberts (12). Manufacturers' directions were followed for the performance of both the Remel Rapid Trehalose Assimilation Broth and the Hardy Diagnostics Trehalose Fermentation Broth with Durham Tube. Both tests require incubation at 42°C, but the Remel Assimilation Broth is incubated for only 3 h, while the Hardy Fermentation Broth is incubated for 24 h. For the Remel Yeast Fermentation Broth with Durham Tube, the manufacturer's directions were modified according to a study by Land et al. (7) that recommends increasing the incubation temperature from 35 to 42°C, overlaying the tubes with mineral oil, and incubating the tubes for 24 h instead of 7 to 24 days. According to Land, the only taxa that ferment trehalose at 42°C are *C. glabrata* and *C. tropicalis. C. tropicalis*, however, is not consistent in fermentation and is larger than *C. glabrata*. The Mayo Clinic Rapid Assimilation Trehalose Broth method was performed as outlined in the Mayo Clinic Mycology Procedure Manual (8). The trehalose broth was prepared in-house according to directions that included 20% yeast nitrogen base, 40% trehalose, bromcresol green (0.02%), and cycloheximide (10,000 μg/ml). Three drops of broth were dispensed into each well of a microtiter plate as needed for tests and controls. A heavy inoculum of yeast was emulsified in the broth of a labeled well. The microtiter plate was incubated for 1 h at 35°C. The Mayo Clinic procedure has been cited in the literature, but to date the medium is not commercially available, and there have been no published studies evaluating it (7, 9). An important comment regarding the inoculum size as described in the procedures for the Mayo Clinic and Remel assimilation tests is that the procedures require either a heavy inoculum or a cloudy suspension. In our experience, this means that the inoculum must be creamy for these tests to work properly.

The guiding criteria for the selection of yeasts to be screened for *C. glabrata* were germ tube negativity, the absence of pseudohyphae in the germ tube, and microscopically small size. From the ARUP laboratory facility, a total of 320 clinical and proficiency sample yeast isolates were tested by all four methods. Among the samples, 119 were archived from a previous yeast study (1) and 201 were recent patient isolates. The samples included 293 *C. glabrata* isolates, 6 *C. lusitaniae* isolates, 5

TABLE 1. Yeast isolates testing positive by screening methodologies

Organism	No. of isolates	No. of isolates testing positive with indicated methodology			
		Mayo Clinic assimilation	Remel assimilation	Remel fermentation	Hardy fermentation
C. glabrata	293	283	268	279	281
C. lusitaniae	6	0	0	2	0
C. parapsilosis	5	1	0	0	0
C. tropicalis	5	2	0	1	0
C. guillermondii	3	1	0	0	0
C. albicans	2	1	1	0	0
C. lipolytica	2	1	0	0	0
S. cerevisiae	2	0	0	0	0
C. krusei	1	1	0	0	0
C. rugosa	1	0	0	0	0

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TABLE 2. Comparative results of four screening methodologies for identification of *C. glabrata*

Test method, no. of hours	No. of results		% Sensitivity	% Specificity
	False negative	False positive		
Mayo assimilation, 1	10	7	96.6	74.1
Remel assimilation, 3	25	1	91.5	96.3
Remel fermentation, 24	14	3	95.0	89.0
Hardy fermentation, 24	12	0	96.0	100

C. parapsilosis isolates, 5 *C. tropicalis* isolates, 3 *C. guillermondii* isolates, 2 *C. albicans* isolates, 2 *C. lipolytica* isolates, 2 *S. cerevisiae* isolates, 1 *C. krusei* isolate, and 1 *C. rugosa* isolate. All 320 isolates were identified by using the API 20C Yeast Identification System (bioMERIEUX, Hazelwood, Mo.) and rice and cornmeal morphology agars. Manufacturer's directions were followed when the API 20C system was used, and morphology agars were streaked according to the Dalmau plate technique (6). The morphology agars were evaluated for the production of chlamydospores, blastoconidia, arthroconidia, pseudohyphae, and true hyphae. It is important that two isolates of *C. glabrata* gave the API 20C profile index number of 2000000, indicating that they did not assimilate trehalose. The profile index number also gave the interpretation of GLLS (good likelihood low selectivity) and then listed the possible identifications as *Blastoschizomyces capitatus*, *C. krusei*, *C. glabrata*, and *C. lambica*. Final identification of these two isolates was done by using the morphology agars. Both isolates tested negative by all four screening methods.

The interpretation of each of the four methodologies for the identification of *C. glabrata* are as follows. For the two assimilation tests, a color change from blue to yellow indicates trehalose utilization. A positive Hardy fermentation test requires the development of gas bubbles in the Durham Tube, with a color change from blue to yellow, while the Remel fermentation test requires only gas development in the Durham Tubes.

Table 1 lists the isolates that tested positive for each rapid screening test. The number of *Candida* species that were not *C. glabrata* and tested positive for the Mayo Clinic Assimilation test is noteworthy. Table 2 shows the sensitivity and specificity of each rapid screening test. Obviously, yeasts other than *C. glabrata* met the initial screening criteria for this study—germ tube negative, no pseudohyphae in the germ tube, and small size. Examples are *C. guillermondii* and *C. lusitaniae*. Theoretically, a screening test should be able to separate *C. glabrata* from these yeasts. This study showed that it is not always easy to determine if a yeast is considered small in size. Even though they are usually considered to be larger yeasts and similar in size to *C. albicans*, isolates of *C. tropicalis*, *C. parapsilosis*, *Saccharomyces cerevisiae*, and *C. krusei* were selected as germ tube-negative yeasts that fit the screening criteria. Two isolates of *C. albicans* were also selected, either because they did not produce germ tubes or because the germ tube test was not correctly performed or interpreted. These findings point out a potential weakness in the protocol which must be considered in comparisons of the specificities of the four screening tests. The highest number of false-positive results was with the Mayo Clinic Assimilation Trehalose Broth. Under the screening protocol, seven isolates were falsely identified as *C. glabrata*. The seven isolates included two *C. tropicalis* isolates, one *C. krusei* isolate, one *C. albicans* isolate, one *C. parapsilosis* isolate, one *C. lipolytica* isolate, and one *C. guillermondii* isolate. In this study, the Mayo Clinic Assimilation Broth was more difficult to interpret because of the various color shades. A recent publication cited difficulties in adjusting the buffer capacity of this test, which is required to avoid false-positive results (9). The low specificity for the Mayo Clinic assimilation test may be significant in terms of choice of antifungal therapy.

The highest number of false-negative results was seen in the Remel Rapid Trehalose Assimilation Broth, which failed to identify 25 of the 283 *C. glabrata* isolates. Following the screening protocol, these 25 isolates were identified by using the API 20C Yeast Identification System in conjunction with the morphology agars. In practice, if a *C. glabrata* isolate tested negative by the rapid trehalose screening procedure, it would then be identified by using the API 20C Yeast Identification System and morphology agars. Also, if a *C. tropicalis* isolate or another isolate tested negative by the screening procedure, as it should, in practice it would be correctly identified by using the API 20C and morphology agars. However, if, for example, a *C. tropicalis* isolate or a *C. parapsilosis* isolate tested positive by the screening procedure, it would be misidentified as a *C. glabrata* isolate. A false-positive result has greater significance in the screen, leading to incorrect identification.

The most impressive results occurred with the Hardy Diagnostics Trehalose Fermentation Broth. The sensitivity was 96%, with a specificity of 100%. However, the drawback of this test is the required 24-h incubation, which precludes its consideration as a rapid method. The other 24-h test, Remel Trehalose Fermentation Broth, had a sensitivity of 95% but a specificity of 89%. The current "gold standard" for yeast identification systems, the API 20C, yields results in 48 to 72 h and also utilizes the morphology plates (1).

Table 3 compares the cost of each methodology, including the API 20C. Certainly the least expensive method ($0.045 per test) involves Mayo Clinic Rapid Assimilation Trehalose Broth. This is based on the use of 96-well break-away microtiter plates and reagent costs. The most expensive screening method is Remel Yeast Fermentation Broth with Durham Tube ($4.15/tube); it costs considerably more than Remel Assimilation Broth ($1.59/tube) and Hardy Diagnostics Trehalose Fermentation Broth ($1.10/tube). All these methods are less costly than the API 20C ($6.60/strip). However, if the rapid trehalose test gives a false-negative result and the isolate is then identified by using the API 20C and morphology agars, the cost is more than that for the API 20C alone.

We recommend the Remel Rapid Trehalose Assimilation Broth method for *C. glabrata* screening. Although this method

TABLE 3. Cost comparison of four screening methods for identifying *C. glabrata* by using list prices

Screening method	Cost ($)
Mayo Clinic Rapid Assimilation Trehalose Broth	0.045
Remel Rapid Trehalose Assimilation Broth	1.59/tube
Remel Yeast Fermentation Broth with Durham Tube	4.15/tube
Hardy Diagnostics Trehalose Fermentation Broth with Durham Tube	1.10/tube
API 20C Yeast Identification System	6.60/test strip

is the least sensitive of the four, it provides identification of this yeast in 3 h and is cost-effective, especially compared to the API 20C Yeast Identification System and the Remel Yeast Fermentation Broth, and the specificity is excellent (96.3%). If a *C. glabrata* isolate tests negative with this method, it will be correctly identified by using the API 20C. Also, this test was the easiest to interpret by all the technical staff who performed the screening protocols. We recommend that the manufacturer of the Remel Rapid Trehalose Assimilation Broth test use a McFarland standard to determine inoculum density.

If a laboratory implements both the 2- to 3-h germ tube test for identification of *C. albicans* and the 3-h trehalose assimilation test for identification of *C. glabrata*, the majority of clinical yeast isolates can be identified within a day of their sufficient growth.

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