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Rapid Identification of *Candida glabrata* with a New Commercial Test, GLABRATA RTT

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The GLABRATA RTT test (Fumouze Diagnostics, Levallois Perret, France) is based on the ability of *Candida glabrata* to hydrolyze trehalose but not maltose. It requires an inoculum of only four to six colonies, and the results are available within 20 min. We tested GLABRATA RTT with 330 stock isolates grown in subcultures on four different primary fungal isolation media and obtained a sensitivity of 94 to 98% (depending on the medium used) and a specificity of 97.3 to 98.6%. The false-positive results corresponded to *C. tropicalis*, *C. famata*, and *C. lusitaniae*. GLABRATA RTT thus offers rapid and reliable identification of *C. glabrata*.

The increasing numbers of immunocompromised patients, and the widespread use of certain medical and surgical practices, are favoring the emergence of normally commensal *Candida* species as life-threatening pathogens. Antifungal susceptibility testing of clinical isolates can take some time, and rapid species-level identification is therefore necessary for prompt initiation of appropriate therapy (23). *Candida glabrata* is one of these emerging pathogens (5, 16, 20, 25). It is the second most commonly isolated yeast species in clinical laboratories (4, 19), and some isolates show decreased susceptibility to several azole antifungal agents, including fluconazole. Clinical laboratories must therefore be capable of rapidly identifying this *Candida* species. Classically, *C. glabrata* identification has been based on biochemical tests (carbohydrate assimilation and fermentation), but these require sufficient biomass and take 24 to 48 h (4, 5, 8).

C. glabrata can be identified more rapidly on the basis of trehalose assimilation or fermentation, but such tests require incubation periods ranging from 1 to 24 h. In addition, the use of pH-based detection requires strict control of buffer capacity (4, 12, 13; L. Stockman and G. Roberts, Program Abstr. 85th Annu. Meet. Am. Soc. Microbiol. 1985, abstr. 377, 1985). Recently, Peltroche-Llacsahuanga et al. (19) and Parant et al. and others (9, 18; F. Parant, M. Crepy, Y. Gille, and A. M. Freydiere, abstr. from the 6th Congress of the European Confederation of Medical Microbiology, Rev. Iberoam. Micol. **17**(Suppl. 132)**:**3, 2000) described a rapid test for *C. glabrata* identification based on a glucose oxidase reaction for the detection of trehalose hydrolysis. This test has the dual advantages of requiring only a small inoculum and a short incubation time. Parant et al. (18) and Freydiere et al. (9) subsequently recommended the addition of a maltose assimilation test to avoid false-positive results with other *Candida* species.

GLABRATA RTT (Fumouze Diagnostics, Levallois Perret, France), the first commercial test for *C. glabrata* identification, takes only 15 to 20 min and is based on the capacity of *C. glabrata* to hydrolyze trehalose but not maltose. It uses a glucose-oxidase reaction to detect glucose generated from trehalose or maltose and requires only a few colonies. A maltose control is used to eliminate false-positive results obtained with other yeasts. Moreover, as most fungal isolation media contain glucose, a carbohydrate-free control is used to detect the possible carryover of glucose-containing agar in the yeast suspension.

The purpose of this study was to assess the performance of this rapid trehalase test for *C. glabrata* identification when applied to colonies grown on four different primary fungal isolation media.

A total of 330 stock isolates representing 16 yeast species commonly encountered in clinical samples (see Table 2) were kept on glass beads at −20°C and grown once in subcultures on Sabouraud agar (Biomerieux, Marcy l'Etoile, France) to check for purity. The 330 isolates were tested in either Lyon (n = 125) or Angers (n = 205). They were streaked to single colonies on the four chromogenic and nonchromogenic media and incubated (for 24 h in Lyon and 48 h in Angers) at 30°C. All the isolates were identified with conventional materials and methods, including germ tube formation, ID 32C identification strips (Biomerieux), morphology on rice agar Tween, and Krusei Color latex agglutination (Fumouze Diagnostics).

We used three chromogenic media (Candida ID [Biomerieux], CHROMagar Candida [Becton Dickinson, Le Pont de Claix, France], and CandiSelect [Bio-Rad, Marnes la Coquette, France]) and one conventional medium (Sabouraud chloramphenicol gentamicin agar; Biomerieux). All of the media were purchased ready to use.

GLABRATA RTT (Fumouze Diagnostics) consists of a panel of 12 wells, permitting the identification of four isolates. Three wells (designated T, M, and B) containing dehydrated medium are required per isolate. In addition to basic medium, wells T and M contain trehalose and maltose, respectively. Following the manufacturer's instructions, four to six colonies are suspended in 100 μl of distilled water (corresponding to a reading of 3 on the McFarland turbidity scale), 25 μl of suspension is added to each well (T, M, and B), and the panel is

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TABLE 1. Interpretation of readings

Test result for well:			Identification of species
T (with trehalose)	M (with maltose)	B (sugar free)	
+	−	−	*C. glabrata*
+	+	−	Non-*C. glabrata*
−	+	−	Non-*C. glabrata*
−	−	−	Non-*C. glabrata*
+ Or −	+ Or −	+	Uninterpretable

incubated for 10 min at room temperature. Glucose formation is detected by adding 25 μl of a reagent mixture of oxidase glucose, peroxidase, and chromogen substrate per well and further incubating the panel for 5 to 10 min at room temperature. A brown-orange color corresponds to the presence of glucose, while a lack of coloration corresponds to a negative result.

The results are shown in Table 1. Only *C. glabrata* strains normally give a positive trehalose and a negative maltose test result, and tests yielding a positive sugar-free control well result are uninterpretable.

Statistical significance was determined by using a paired *t* test, and *P* values of <0.05 were considered significant.

We evaluated the performance of GLABRATA RTT by testing 330 yeast isolates, consisting of 100 *C. glabrata* strains and 230 non-*C. glabrata* strains, as identified using conventional methods. As the composition of the medium used to isolate a yeast may influence physiological status and, possibly, enzyme expression (18), the results of yeast identification methods based on enzyme reactions may differ according to the medium used. We examined the potential influence of the culture medium on GLABRATA RTT performance by growing subcultures of the 330 stock isolates on the three most commonly used chromogenic media (Candida ID, CHROMagar Candida, and CandiSelect) and one conventional medium (Sabouraud chloramphenicol gentamicin agar) prior to testing.

The sensitivity of GLABRATA RTT ranged from 94 to 98% (depending on the medium), while specificity ranged from 97.3 to 98.6% (Table 2). The false-positive results (1.4 to 2.7% of the 330 isolates) corresponded to *C. tropicalis*, *C. famata*, and *C. lusitaniae.*

GLABRATA RTT gave results that were consistent between the two laboratories participating in this study. Sensitivity ranged from 94.4% ± 3.1% to 98.1% ± 1.8% in Lyon and from 94.4% ± 3.1% to 98.6% ± 1.7% in Angers, and the respective specificity values were 93.4% ± 2.9% to 100% ± 0.3% and 98% ± 1.1% to 99% ± 0.8%; no significant differences were observed between the laboratories regardless of the culture medium used.

GLABRATA RTT is simple to use and yields results within 20 min with an inoculum of only four to six colonies, making it the most rapid commercial *C. glabrata* identification test. Some of the *C. glabrata* strains that gave a false-negative trehalase test result gave a positive result on secondary testing, suggesting that an inadequate inoculum had been used in the first test. Other rare strains were not identified as *C. glabrata* because the results were uninterpretable. As Freydiere et al. have previously reported (9), the addition of the maltose control reduces the number of false-positive results and increases the specificity of GLABRATA RTT to more than 97%, which is excellent for a phenotype-based identification test.

In contrast to other studies (18, 19), we did not find that the results of the rapid trehalase test differed according to the growth medium used. Indeed, neither sensitivity nor specificity differed significantly among the four media used.

Depending on the medium used for primary culture of clinical specimens, the following two diagnostic algorithms can be proposed for identification of *C. albicans* and *C. glabrata*, which together account for about 80% of clinical isolates (of those isolates, about 60% are *C. albicans* and 20% are *C. glabrata*). (i) After primary culture on Sabouraud agar, rapid *C. albicans* identification can be obtained with the germ tube test (requiring 2 to 4 h) or the Bichro-latex albicans agglutination test (Fumouze Diagnostics) (5 min) (6, 21, 22). Colonies that are not positively identified with these two methods can be tested with GLABRATA RTT. (ii) After primary growth in cultures on a chromogenic medium, which permits rapid identification and/or group classification of *Candida* species on the basis of their characteristic colony colors, only colonies showing the characteristic color of the *C. glabrata* group need to be tested with GLABRATA RTT. Many laboratories now use commercial chromogenic media such as Candida ID, CandiSelect, and CHROMagar Candida. Candida ID and CandiSelect identify *C. albicans* colonies on the basis of their char-

TABLE 2. Results of the GLABRATA RTT test on 330 yeast strains grown in subcultures on three chromogenic agar media and on Sabouraud gentamicin chloramphenicol agar

Strain	No. of strains tested (n = 330)	No. of strains identified as *C. glabrata*[a] with the GLABRATA RTT test according to the indicated subculture medium			
		Candida ID (Biomerieux)	CHROMagar Candida (Becton Dickinson)	CandiSelect (Bio-Rad)	Sabouraud chloramphenicol gentamicin agar (Biomerieux)
Candida glabrata	100	98	94	98	98
Non-*Candida glabrata* species	230	5	3	6	3
Candida tropicalis	42	2	1	1	1
Candida famata	17	2	2	4	2
Candida lusitaniae	14	1	0	1	0
Other species[b]	157	0	0	0	0

[a] *C. glabrata* strains are trehalase positive and maltase negative. Sensitivity values for Candida ID, CHROMagar Candida, CandiSelect, and Sabouraud chloramphenicol gentamicin agar were 98, 94, 98, and 98%, respectively; specificity values were 97.8, 98.6, 97.3, and 98.6%, respectively.

[b] *C. albicans* (n = 30), *C. parapsilosis* (n = 28), *C. krusei* (n = 28), *C. dubliniensis* (n = 18), *C. guilliermondii* (n = 16), *Saccharomyces cerevisiae* (n = 14), *C. kefyr* (n = 10), *C. inconspicua* (n = 4), *C. lipolytica* (n = 3), *C. norvegensis* (n = 3), *C. pelliculosa* (n = 2), and *C. utilis* (n = 1).

acteristic blue color. Candida ID also contains a second substrate allowing the classification of several species (not including *C. glabrata*) in a group yielding pink colonies (7, 10, 24). *C. glabrata* yields white colonies on these two media, and thus, only these colonies need to be tested with GLABRATA RTT. CHROMagar Candida permits the identification of *C. albicans*, *C. tropicalis*, and *C. krusei* (1, 11, 17; A. M. Freydiere, Letter, J. Clin. Microbiol. **34:**2048, 1996) on the basis of their colony colors, and *C. glabrata* is included in the group of pink colonies along with several other species (Freydiere, letter). Thus, when this medium is used for primary isolation, only pink colonies need to be tested with GLABRATA RTT.

In this study, the sensitivity and specificity of GLABRATA RTT for *C. glabrata* exceeded 94%, regardless of the primary isolation medium. These results match those achieved with slower trehalase-based tests (4, 12, 19). Widely used commercial yeast identification systems such as API20CAUX, Auxacolor, Fungichrom, and Vitek YBC often perform less well than GLABRATA RTT (2, 8).

Recent studies have shown that multiplex PCR-based methods (3, 14) and vibrational spectrometry techniques such as Fourier transform infrared spectroscopy and Raman spectroscopy (15) are rapid, sensitive, and accurate for species-level identification of *Candida* isolates. However, although some of these methods (15) can identify *Candida* spp. with 6-h agar microcolonies, their use is restricted to large reference and research laboratories because of their high cost and the need for specialized equipment. Unless these methods can be adapted for routine use in clinical microbiological laboratories, rapid phenotypic tests are likely to remain the most cost-effective approach.

In conclusion, this first study performed with stock isolates shows that GLABRATA RTT reliably identifies *C. glabrata* grown in subcultures on the three most commonly used chromogenic media (Candida ID, CandiSelect, and CHROMagar Candida). Further evaluation will be necessary to determine (i) the utility of GLABRATA RTT in routine use, (ii) interlaboratory reproducibility, and (iii) potential geographical phenotypic variations among clinical isolates. We are currently testing GLABRATA RTT on fresh isolates in the routine clinical setting in a study involving several medical mycology laboratories in different countries.

REFERENCES

1. **Baumgartner, C., A.-M. Freydiere, and Y. Gille.** 1996. Direct identification and recognition of yeast species from clinical material by using Albicans ID and CHROMagar Candida plates. J. Clin. Microbiol. **34:**454–456.
2. **Buchaille, L., A. M. Freydiere, R. Guinet, and Y. Gille.** 1998. Evaluation of six commercial systems for identification of medically important yeasts. Eur. J. Clin. Microbiol. Infect. Dis. **17:**479–488.
3. **Chang, H. C., S. N. Leaw, A. H. Huang, T. L. Wu, and T. C. Chang.** 2001. Rapid identification of yeast in positive blood cultures by a multiplex PCR method. J. Clin. Microbiol. **39:**3466–3471.
4. **Fenn, J. P., E. Billetdeaux, H. Segal, L. Skodack-Jones, P. E. Padilla, M. Bale, and K. Carroll.** 1999. Comparison of four methodologies for rapid and cost-effective identification of *Candida glabrata.* J. Clin. Microbiol. **37:**3387–3389.
5. **Fidel, P. L., J. A. Vazquez, and J. D. Sobel.** 1999. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans.* Clin. Microbiol. Rev. **12:**80–96.
6. **Freydiere, A.-M., L. Buchaille, R. Guinet, and Y. Gille.** 1997. Evaluation of latex reagents for rapid identification of *Candida albicans* and *C. krusei* colonies. J. Clin. Microbiol. **35:**877–880.
7. **Freydiere, A. M., F. Parant, C. Chaux, and Y. Gille.** 2000. Candida ID, a new chromogenic medium compared to albicans ID2. Clin. Microbiol. Infect. **6:**181.
8. **Freydiere, A. M., R. Guinet, and P. Boiron.** 2001. Yeast identification in the clinical microbiology laboratory: phenotypical methods. Med. Mycol. **39:**9–33.
9. **Freydière, A.-M., F. Parant, F. Noel-Baron, M. Crepy, A. Treny, H. Raberin, A. Davidson, and F. C. Odds.** 2002. Identification of *Candida glabrata* by a 30-second trehalase test. J. Clin. Microbiol. **40:**3602–3605.
10. **Fricker-Hidalgo, H., S. Orenga, B. Lebeau, H. Pelloux, M. P. Brenier-Pinchart, P. Ambroise-Thomas, and R. Grillot.** 2001. Evaluation of Candida ID, a new chromogenic medium for fungal isolation and preliminary identification of some yeast species. J. Clin. Microbiol. **39:**1647–1649.
11. **Jabra-Rizk, M. A., T. M. Brenner, M. Romagnoli, A. A. M. A. Baqui, W. G. Merz, W. A. Falkler, Jr., and T. F. Meiller.** 2001. Evaluation of a reformulated CHROMagar Candida. J. Clin. Microbiol. **39:**2015–2016.
12. **Land, G., J. Burke, C. Shelby, J. Rhodes, J. Collett, I. Bennett, and J. Johnson.** 1996. Screening protocol for *Torulopsis* (*Candida*) *glabrata.* J. Clin. Microbiol. **34:**2300–2303.
13. **Lopez, J., F. Dalle, P. Mantelin, P. Moiroux, A. C. Nierlich, A. Pacot, B. Cuisenier, O. Vagner, and A. Bonnin.** 2001. Rapid identification of *Candida glabrata* based on trehalose and sucrose assimilation using Rosco diagnostic tablets. J. Clin. Microbiol. **39:**1172–1174.
14. **Luo, G., and T. G. Mitchell.** 2002. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. J. Clin. Microbiol. **40:**2860–2865.
15. **Maquelin, K., L.-P. Choo-Smith, H. P. Endtz, H. A. Bruining, and G. J. Puppels.** 2002. Rapid identification of *Candida* species by confocal Raman microspectroscopy. J. Clin. Microbiol. **40:**594–600.
16. **Michel-Nguyen, A., A. Favel, P. Azan, P. Regli, and A. Penaud.** 2000. Dix-neuf années de données épidémiologiques en centre hospitalier universitaire: place de *Candida* (*Torulopsis*) *glabrata*; sensibilité. J. Mycol. Med. **10:**49–51.
17. **Odds, F. C., and R. Bernaerts.** 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32:**1923–1929.
18. **Parant, F., A. M. Freydiere, Y. Gille, P. Boiron, and F. C. Odds.** 2001. A one-minute trehalase detection test for identification of *Candida glabrata.* J. Mycol. Med. **11:**26–31.
19. **Peltroche-Llacsahuanga, H., N. Schnitzler, R. Lutticken, and G. Haase.** 1999. Rapid identification of *Candida glabrata* by using a dipstick to detect trehalase-generated glucose. J. Clin. Microbiol. **37:**202–205.
20. **Price, M. F., M. T. La Rocco, and L. O. Gentry.** 1994. Fluconazole susceptibilities of *Candida* species and distribution of species recovered from blood cultures over a 5-year period. Antimicrob. Agents Chemother. **38:**1422–1424.
21. **Quindos, G., R. San Millan, R. Robert, C. Bernard, and J. Ponton.** 1997. Evaluation of Bichro-latex albicans, a new method for rapid identification of *Candida albicans.* J. Clin. Microbiol. **35:**1263–1265.
22. **Robert, R., R. Sentandreu, C. Bernard, and J.-M. Senet.** 1994. Evaluation du réactif Bichrolatex albicans pour l'identification de colonies de *Candida albicans.* J. Mycol. Med. **4:**226–229.
23. **Rowen, J. L., J. M. Tate, N. Nordoff, L. Passarell, and M. R. McGinnis.** 1999. *Candida* isolates from neonates: frequency of misidentification and reduced fluconazole susceptibility. J. Clin. Microbiol. **37:**3735–3737.
24. **Willinger, B., C. Hillowoth, B. Selitsch, and M. Manafi.** 2001. Performance of Candida ID, a new chromogenic medium for presumptive identification of *Candida* species, in comparison to CHROMagar Candida. J. Clin. Microbiol. **39:**3793–3795.
25. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, C. B. Miller, J. E. Karp, and R. Saral.** 1993. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patient. Antimicrob. Agents Chemother. **37:**1847–1849.