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

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This study compares the performance of a 3-h dipstick trehalose test with GLABRATA RTT, a new commercially available 20-min test for the rapid identification of *Candida glabrata*. With the exception of blood agar, GLABRATA RTT gave reliable results with all media tested and was always superior to the dipstick test.

The incidence of fungal infections has increased steadily over the past decade, due in part to the increase in *Candida* species other than *C. albicans*. *Candida glabrata* is now reported to be the second most frequently isolated yeast species from clinical infections after *C. albicans* (2). In a recent surveillance program, *C. glabrata* was reported to cause 17 to 23% of cases of bloodstream infections (11). Since candidemia is associated with a high mortality rate, prompt appropriate antifungal therapy is essential. Due to the commonly occurring innate or acquired resistance of *C. glabrata* to fluconazole, rapid identification is essential. Therefore, several groups have tried to establish protocols for screening and rapid identification of this yeast (3–10).

The aim of the present study was to compare the performance of a dipstick test described by Peltroche-Llacsahuanga (9) and the new, commercially available GLABRATA RTT test (Fumouze Diagnostics, Levallois-Perret Cedex, France) for rapid identification of *C. glabrata*. Both tests are based on rapid hydrolysis of trehalose. Although this enzyme is frequently encountered in other species of yeasts, it is claimed that none of these species hydrolyzes trehalose as rapidly as *C. glabrata* (1). For GLABRATA RTT, both a maltose test and a control test are included to improve specificity.

A total of 332 yeast strains were identified by conventional methods as *C. albicans* ($n = 109$), *C. glabrata* ($n = 90$), *C. tropicalis* ($n = 40$), *C. parapsilosis* ($n = 38$), *C. krusei* ($n = 18$), *C. guilliermondii* ($n = 9$), *C. dubliniensis* ($n = 8$), *C. kefyr* ($n = 6$), *C. pelliculosa* ($n = 4$), *C. lusitaniae* ($n = 3$), *C. rugosa* ($n = 2$), *C. sake* ($n = 2$), *C. utilis* ($n = 1$), *Candida* sp. ($n = 1$), and *Saccharomyces cerevisiae* ($n = 1$). These isolates were subcultured by streaking single colonies on Chromagar Candida (Becton Dickinson, Franklin Lakes, N.J.), Candida ID agar (bioMérieux, Marcy l'Etoile, France), Sabouraud agar containing 4% glucose (Oxoid, Basingstoke, United Kingdom), Sabouraud agar containing 2% glucose (Oxoid, Basingstoke, United Kingdom), and Columbia blood agar base supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated for 24 h at 35°C. As GLABARATA RTT had not been evaluated previously with strains grown on blood agar routinely used in microbiology laboratories, this medium was also included.

Both tests for rapid identification of *C. glabrata* were then performed simultaneously without the investigator's knowledge of the exact species. For the GLABRATA RTT, 25 μl of the yeasts suspended in distilled water was put into each of three wells containing trehalose, maltose, and sugar-free basic medium, which served as a control. After incubation for 10 min at room temperature, 25 μl of a revealing reagent containing glucose oxidase, peroxidase, and a chromogenic substrate was added to each of the wells. After another incubation for 5 to 10 min at room temperature, the results were read. Whenever an orange color developed only in the well containing trehalose, the yeast was identified as *C. glabrata*. If other wells also gave positive reactions, the result was interpreted as a yeast, not *C. glabrata*. For the dipstick test described by Peltroche-Llacsahuanga, one colony of each yeast strain was suspended in 50 μl of citrate buffer containing 4% (wt/vol) trehalose (Merck, Darmstadt, Germany) for 3 h at 37°C. Glucose generated by cleavage due to cell-bound trehalase was detected with a commercially available dipstick test (Diabur-test 5000; Boehringer, Mannheim, Germany) by assessing color change from yellow to green. Thus, the isolate was interpreted as *C. glabrata*.

In the first step, the results of both tests obtained on nonchromogenic and chromogenic media were analyzed (Table 1). For the GLABRATA RTT, depending on the medium, 91.1 to 96.7% of the 90 *C. glabrata* strains gave positive results, whereas 0.4 to 16.1% of the 242 non-*C. glabrata* strains gave false-positive results. The rate of false-positive results was notably higher with strains grown on BA. For the dipstick test, depending on the medium, 67.8 to 97.8% of the 90 *C. glabrata* strains gave a positive result, whereas 10.7 to 42.6% of the 242 non-*C. glabrata* strains gave false-positive results. Similar to the GLABRATA RTT, the rate of false-positive results was again notably higher with strains grown on BA.

Table 2 shows the number and species of non-*C. glabrata* isolates yielding false-positive results with the GLABRATA RTT and the dipstick test on the different media. Regardless of the medium, the rate of false-positive results was always higher with the dipstick test. The highest rate of false-positive results

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TABLE 1. Comparison of the GLABRATA RTT test (RTT) and the dipstick test, described by Peltroche-Llacsahuanga et al. (DT) for the rapid identification of *Candida glabrata*

Parameter[a]	No. of strains tested	No. of strains identified as *C. glabrata*[b]									
		Chromagar Candida		Candida ID		SAB 4%		SAB 2%		Blood agar	
		RTT	DT	RTT	DT	RTT	DT	RTT	DT	RTT	DT
C. glabrata	90	87	61	86	88	82	81	87	82	84	86
Non-*C. glabrata*	242	1	28	5	77	1	26	4	34	39	103
Sensitivity (%)		96.7	67.8	95.6	97.8	91.1	90	96.7	91.1	93.3	95.6
Specificity (%)		99.6	88.4	97.9	68.2	99.6	89.3	98.3	86.0	83.9	57.4
PPV (%)		98.9	68.5	94.5	53.3	98.8	75.7	95.6	70.7	68.3	45.5
NPV (%)		98.8	88.1	98.3	98.8	96.4	96.2	98.8	96.7	97.1	97.2
Efficiency (%)		98.8	82.8	97.3	76.2	97.3	89.5	97.9	87.4	86.5	67.8

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] SAB, Sabouraud with 4 or 2% glucose.

was obtained with *C. tropicalis*, ranging from 25.5% (Sabouraud with 2% glucose) up to 77.5% (Candida ID) with the dipstick test compared to only one strain with the GLABRATA RTT. In contrast to the results of Peltroche-Llacsahuanga, we found that species other than *C. tropicalis* and *C. albicans* could also be misidentified with the dipstick test. For example, *C. guilliermondii* showed a high rate of false-positive results on Candida ID (66.7%) and Sabouraud with 4% glucose (55.6%). The higher specificity of the GLABRATA RTT is related to the inclusion of both a maltose test and a sugar-free control, as previously reported (5, 6), and thus rules out other *Candida* spp., e.g., *C. tropicalis*, which sometimes yield a positive trehalose result. The sugar-free control is designed to eliminate false-positive results due to carryover of extraneous glucose with the yeast inoculum.

For both the GLABRATA RTT and the dipstick test, the use of media containing blood resulted in an unacceptably high rate of false-positive reactions, mainly due to *C. tropicalis* and *C. albicans* strains. This point had been previously shown by Peltroche-Llacsahuanga et al. (9), who showed that up to 68.5% of *C. tropicalis* and 2% of *C. albicans* gave false-positive results. The reason for these false-positive results remains unclear but might be the reaction of blood with peroxidase, which is a constituent of the detection system. GLABRATA RTT has not been evaluated before with strains grown on blood agar. Therefore, yeasts cultivated on media supplemented with blood cannot be recommended for the rapid identification of *Candida glabrata* with either the GLABRATA RTT or dipstick test.

TABLE 2. Number and species of non-*C.glabrata* isolates yielding a false-positive result with the GLABRATA RTT test (RTT) and the dipstick test (DT) after growth on the indicated agar[a]

Species	No. of strains tested	No. of strains identified									
		Chromagar Candida		Candida ID		SAB 4%		SAB 2%		Blood agar	
		RTT	DT	RTT	DT	RTT	DT	RTT	DT	RTT	DT
C. albicans	109	0	3	0	30	1	3	0	15	20	49
C. tropicalis	40	1	22	0	31	0	12	0	11	9	35
C. parapsilosis	38	0	2	0	3	0	3	1	6	0	6
C. guilliermondii	9	0	0	2	6	0	5	0	1	6	8
C. kefyr	6	0	0	0	0	0	0	1	1	0	0
C. lusitaniae	3	0	0	2	2	0	0	1	0	3	3
C. sake	2	0	0	1	1	0	0	1	0	1	1
C. krusei	18	0	0	0	1	0	1	0	0	0	1
C. dubliniensis	8	0	1	0	1	0	0	0	0	0	0
C. rugosa	2	0	0	0	2	0	2	0	0	0	0
Other yeasts[b]	7	0	0	0	0	0	0	0	0	0	0
Total	242	1	28	5	77	1	26	4	34	39	103

[a] See Table 1, footnote *b*.
[b] *C. pelliculosa* ($n = 4$), *C. utilis* ($n = 1$), *C. species* ($n = 1$), and *Saccharomyces cerevisiae* ($n = 1$).

TABLE 3. Comparison of the GLABRATA RTT test (RTT) and the dipstick test (DT) with isolates resembling *C. glabrata* on Chromagar Candida ($n = 157$) and Candida ID ($n = 133$)

Parameter[a]	Positive result on Chromagar Candida by:		Positive result on Candida ID by:	
	RTT	DT	RTT	DT
C. glabrata ($n = 90$)	87	61	86	88
Non-*C. glabrata*[b]	0	2	0	5
Sensitivity (%)	96.7	67.8	95.6	97.8
Specificity (%)	100	97.0	100	88.4
PPV (%)	100	96.8	100	94.6
NPV (%)	95.7	69.2	91.5	95.0
Efficiency (%)	98.1	80.3	97.0	94.7

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] $n = 67$ for Chromagar and 43 for Candida ID.

As chromogenic media allow identification of certain *Candida* species, e.g., *C. albicans* and *C. tropicalis*, by means of their species-specific color, in a second step, we selected strains resembling *C. glabrata* on Chromagar Candida and Candida ID agar for further evaluation. Thus, 157 strains showing pink colonies on Chromagar Candida and 133 strains showing white colonies on Candida ID agar were selected for further analysis. Table 3 shows the results of both tests performed with suspected strains of *C. glabrata* and demonstrates that the performance of both rapid tests is improved when chromogenic media are used. Our results show that both chromogenic media are convenient for further trehalase testing with GLABRATA RTT. This is also true for the dipstick test, and here the best performance was achieved on Candida ID, though it still differs significantly (Fisher's exact test; $P > 0.05$) from the results obtained with GLABRATA RTT on this medium.

In conclusion, this study clearly demonstrates the high accuracy of GLABRATA RTT for the presumptive identification of *C. glabrata* and the superiority to the dipstick test. However, neither the GLABRATA RTT nor the dipstick test showed acceptable performance with colonies grown on blood agar. If a laboratory uses both a chromogenic medium and the GLABRATA RTT for identification of *C. glabrata*, the majority of clinical isolates can be reliably identified within 20 min provided that sufficient growth is available for testing.

REFERENCES

1. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 1990. Yeasts: characteristics and identification, 2nd ed., p. 167. Cambridge University Press, Cambridge, Mass.
2. **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.
3. **Freydiere, A. M., R. Guinet, and P. Boiron.** 2001. Yeast identification in the

clinical microbiology laboratory: phenotypical methods. Med. Mycol. **39:**9–33.

4. **Freydiere, 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.
5. **Freydiere, A. M., R. Robert, C. Ploton, A. Marot-Leblond, F. Monerau, and F. Vandenesch.** 2003. Rapid identification of *Candida glabrata* with a new commercial test, GLABRATA RTT. J. Clin. Microbiol. **41:**3861–3863.
6. **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.
7. **Lopez, J., F. Dalle, P. Mantelin, P. Moiroux, A. C. Nierlich, A. Pacot, B. Cuisenier, O. Vagner, and A. Bonnin.** 2001. Rapid identification of *C. glabrata* based on trehalose and sucrose assimilation using Rosco diagnostic tablets. J. Clin. Microbiol. **39:**1172–1174.
8. **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.
9. **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.
10. **Peltroche-Llacsahuanga, H., A. Jenster, R. Lütticken, and G. Haase.** 1999. Novel microtiter plate format for testing germ tube formation and proposal of a cost-effective scheme for yeast identification in a clinical laboratory. Diagn. Microbiol. Infect. Dis. **35:**197–204.
11. **Pfaller, M. A., R. N. Jones, G. V. Doern, H. S. Sader, S. A. Messer, A. Houston, S. Coffman, R. J. Hollis, and the SENTRY Participant Group (Europe).** 2000. Bloodstream infections due to *Candida* species: SENTRY Antimicrobial Surveillance Program in North America and Latin America, 1997–1998. Antimicrob. Agents Chemother. **44:**747–751.