Journal of Clinical Microbiology, Oct. 2004, p. 4870–4872 Vol. 42, No. 10
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.10.4870–4872.2004
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Routine Use of a Commercial Test, GLABRATA RTT, for Rapid Identification of *Candida glabrata* in Six Laboratories

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Received 30 March 2004/Returned for modification 10 May 2004/Accepted 6 July 2004

When evaluated in six clinical laboratories from six countries with 1,174 fresh isolates, including 715 *Candida glabrata* and 459 non-*C. glabrata* strains, GLABRATA RTT (Fumouze Diagnostics, Levallois Perret, France) yielded an overall sensitivity and an overall specificity of 95.8 and 98.9%, respectively. The results were consistent from one laboratory to another. The five false-positive results corresponded to *C. parapsilosis* (*n* = 2), *C. tropicalis*, *C. guilliermondii*, and *C. lusitaniae*. GLABRATA RTT allows a rapid, cost-effective, and reliable presumptive identification of *C. glabrata*.

Candida glabrata emerged as an important opportunistic pathogen in the 1990s and is one of the most commonly encountered pathogenic yeast species after *Candida albicans* (2, 4). Moreover, *C. glabrata* infection is associated with a high rate of morbidity and mortality in immunocompromised patients (11, 19, 21), and some strains show decreased susceptibility to azole antifungal agents, including fluconazole (14, 16, 18). Since antifungal susceptibility testing can take some time, rapid species-level identification is important for prompt initiation of appropriate therapy.

To facilitate the identification of yeasts in clinical laboratories, several chromogenic media have been introduced that allow immediate identification of *C. albicans* (6, 9, 20) and may identify other important species, such as *Candida tropicalis* and *Candida krusei*, by their colonial appearance (1, 12). However, it has been clearly demonstrated by several authors (5, 12, 20) that no chromogenic medium is yet available which can provide specific identification of *C. glabrata.*

The GLABRATA RTT test (Fumouze Diagnostics, Levallois Perret, France) is a new commercially available test detecting *C. glabrata* within 20 min. It is based on the ability of *C. glabrata* to hydrolyze trehalose and includes both a maltose test and a control test to improve the specificity of the test as previously recommended (7, 15). In a preliminary study with 330 stock isolates, GLABRATA RTT reliably identified *C. glabrata* subcultured on three commonly used chromogenic media: Candida ID, CandiSelect, and CHROMagar Candida (8). The present evaluation was designed to determine the utility of GLABRATA RTT for identification of fresh *C. glabrata* isolates from primary isolation media. The evaluation was performed in six countries to examine interlaboratory reproducibility and potential phenotypic variation among strains from different geographical locations.

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From September 2002 to July 2003, 1,174 yeast isolates, isolated from 1,134 various clinical specimens, were prospectively tested in the Medical Parasitology-Mycology Laboratory, Grenoble, France; the Microbiology Laboratory of the Medical University, Vienna, Austria; the Medical Parasitology-Mycology Laboratory, Milan, Italy; the Microbiology Laboratory, Newcastle upon Tyne, United Kingdom; the Medical Parasitology-Mycology Laboratory, Valencia, Spain; and the Microbiology Laboratory, Nijmegen, The Netherlands. The six laboratories were randomly coded as labs A, B, C, D, E, and F.

Depending on the laboratory, the specimens were subcultured on different primary isolation media, including chromogenic and nonchromogenic media (Table 1). The plates were incubated for 48 h at 37°C for chromogenic media or at 30°C for Sabouraud agar.

On chromogenic media, only strains resembling *C. glabrata* were selected for further evaluation. *C. glabrata* forms pink colonies on CHROMagar Candida and white colonies on Albicans ID2 and Candida ID agar. On Sabouraud agar, in most of the cases, only the colonies yielding negative germ tube test results were tested with the GLABRATA RTT test.

GLABRATA RTT was used in accordance with the manufacturer's instructions.

All isolates were also identified by conventional methods, including evaluation of germ tube formation, morphology on rice agar-Tween, and assimilation pattern by use of (i) ID 32C identification strips (bioMérieux, Marcy l'Etoile, France) for labs A, B, and C; (ii) Vitek 2 (bioMérieux) for lab E; and (iii) Auxacolor (Bio-Rad, Marnes la Coquette, France) for labs D and F. In addition, lab A used the latex agglutination Krusei-Color test (Fumouze) for the identification of *C. krusei*, and lab C used the Candida Check antiserum (Iatron Laboratories, Tokyo, Japan) for the identification of *Candida* species.

Fisher's exact test was used for categorical variables. Differences between groups were considered to be significant when P values were <0.05.

Using conventional methods, the 1,174 yeast isolates were identified as follows: *C. glabrata* (*n* = 715), *Candida parapsi-*

TABLE 1. GLABRATA RTT test results for a total of 1,174 yeast isolates tested in six laboratories from six countries[a]

Parameter	All laboratories, all media	Lab A, Candida ID	Lab B, CHROMagar Candida	Lab C, Sabouraud–chloramphenicol–4% dextrose	Lab D, Albicans ID2	Lab E, CHROMagar Candida	Lab F, Sabouraud–chloramphenicol–4% dextrose
Candida glabrata	685 (715)	113 (114)	133 (138)	163 (165)	98 (102)	127 (129)	51 (67)
Other yeasts	5 (459)	1[b] (91)	0 (58)	0 (103)	3[c] (75)	1[b] (39)	0 (93)
Sensitivity	95.8	99.1	96.4	98.1	96.1	98.4	76.1
Specificity	98.9	98.9	100	100	96	97.4	100

[a] Yeast data are numbers of positive GLABRATA RTT tests (with the number of strains tested shown in parentheses) obtained in each laboratory with yeast colonies grown on the indicated agar. Sensitivity and specificity values are percentages. The sources of the agars used in labs A, B, C, D, E, and F, respectively, were as follows: bioMèrieux, Marcy l'Etoile, France; Becton Dickinson, le Pont de Claix, France; Becton Dickinson GmbH, Heidelberg, Germany; bioMèrieux; Izasa, Barcelona, Spain; and Oxoid LTD, Basingstoke, Hampshire, United Kingdom.

[b] One *C. parapsilosis* isolate.

[c] One *C. tropicalis* isolate, one *C. lusitaniae* isolate, and one *C. guilliermondii* isolate.

losis (n = 112), *C. albicans* (n = 51), *C. tropicalis* (n = 62), *Candida kefyr* (n = 27), *C. krusei* (n = 36), *Candida inconspicua* (n = 7), *Candida lusitaniae* (n = 30), *Candida guilliermondii* (n = 17), *Candida rugosa* (n = 7), *Candida famata* (n = 6), *Candida spherica* (n = 1), *Candida pelliculosa* (n = 4), *Candida norvegensis* (n = 6), *Candida colliculosa* (n = 1), *Candida melibiosica* (n = 1), *Candida dubliniensis* (n = 1), other *Candida* spp. (n = 2), *Saccharomyces cerevisiae* (n = 64), *Cryptococcus* spp. (n = 2), *Trichosporon* spp. (n = 2), *Geotrichum* spp. (n = 12), *Rhodotorula* spp. (n = 5), *Hansenula* spp. (n = 1), *Kloeckera japonica* (n = 1), and *Dekkera bruxellensis* (n = 1).

Table 1 shows that the GLABRATA RTT test allowed the identification of 685 of 715 *C. glabrata* strains (sensitivity, 95.8%), and only 5 of 459 isolates of other yeast species yielded false-positive results (specificity, 98.9%). For five of the six laboratories using different primary isolation media, the sensitivity varied from 96.1 to 99.1%, and the specificity varied from 96 to 100%. In laboratory F, the sensitivity was 76.1%, and the specificity was 100%. However, due to the lower number of *C. glabrata* strains (67 versus >100 strains) tested in this laboratory, the statistical analysis of the sensitivity and specificity results showed that the differences between the six laboratories were not statistically significant ($P > 0.05$).

Of the 30 unidentified *C. glabrata* isolates, 19 yielded uninterpretable results (due to a positive sugar-free control), nine yielded negative trehalose results (due to a too-low inoculum density as demonstrated by retesting), and two yielded positive results for both trehalose and maltose. The 19 uninterpretable results were obtained in laboratories C (n = 1) and F (n = 15) using Sabouraud–chloramphenicol–4% dextrose agar and in laboratory B (n = 3) using CHROMagar Candida. Tests could be performed directly from the primary isolation medium in 95% of cases, thus reducing the time needed for identification. This allowed for an informed decision regarding the most appropriate antifungal treatment to be made at an earlier stage.

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. Previously evaluated with 330 stock isolates subcultured on three chromogenic media and Sabouraud-chloramphenicol agar, GLABRATA RTT had sensitivity and specificity rates for *C. glabrata* in excess of 94%, regardless of the isolation medium (8). In this study, GLABRATA RTT was capable of identifying fresh *C. glabrata* isolates with a sensitivity of 95.8% and a specificity of 98.9%.

Uninterpretable results had previously been explained by the carryover of glucose-containing agar in the yeast suspension (7). However, laboratories C and F, both using Sabouraud-chloramphenicol agar with 4% dextrose, obtained rates of uninterpretable results of 0.9 and 22.4%, respectively. In fact, most of the uninterpretable results were found in the first weeks of use of the test, when the staff were not familiar with this test.

For yeast identification, it could be argued that specificity is more important than sensitivity, particularly for a rapid screening test. Occasionally missing a *C. glabrata* isolate in a rapid presumptive test is not a serious problem, since the isolate should subsequently be identified correctly with more elaborate conventional methods. However, misidentifying a yeast isolate could have serious clinical consequences (17). An analysis of the data reveals the value of the maltose test and the sugar-free control in the GLABRATA RTT test, as both of these enhanced the specificity of *C. glabrata* identification. A total of 47 non-*C. glabrata* strains hydrolyzed trehalose but were excluded as *C. glabrata* due to a positive maltose test. The inclusion of the maltose test therefore increased the overall test specificity from 92.3 to 98.9%. Similarly, the sugar-free control test was just as valuable, as 53 non-*C. glabrata* strains gave false-positive reactions due to carryover of extraneous glucose with the yeast inoculum.

The results of this study show consistently high sensitivity and specificity from one laboratory to another, and this confirms the good interlaboratory reproducibility previously found between two laboratories (8). The sensitivity and specificity also compare well with those achieved with slower trehalase-based tests (3, 10, 13).

Direct recognition of *C. albicans* on chromogenic media, coupled with the GLABRATA RTT test for colonies resembling *C. glabrata*, allowed for rapid presumptive identification of the two yeast species most commonly encountered in clinical samples. Moreover, the use of chromogenic media limited the amount of testing required to identify suspect colonies of *C. glabrata*, thus reducing turnaround time and reagent usage.

In conclusion, GLABRATA RTT is highly effective for presumptive identification of *C. glabrata* in a routine clinical laboratory setting with strains isolated either on Sabouraud-chloramphenicol agar or on one of three commonly used chromogenic media and yields consistent results between different laboratories in different countries.

We thank Fumouze Diagnostics for graciously providing their GLABRATA RTT test. We gratefully thank the technicians from the six laboratories for their technical assistance.

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