

ORIGINAL ARTICLE

Routine use of a one minute trehalase and maltase test for the identification of *Candida glabrata* in four laboratories

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Aims: To evaluate the rapid identification of *Candida glabrata* using a one minute trehalase and maltase test in four clinical laboratories.

Method: The test was evaluated with 944 freshly isolated yeasts comprising 572 *C glabrata* and 372 non-*C glabrata* strains. These strains were isolated on one of three differential media—Candida ID, CHROMagar Candida, or Albicans ID2 medium—and all strains were fully identified using standard methods.

Results: The trehalase and maltase test allowed the overall identification of 550 of 572 *C glabrata* strains (sensitivity, 96.2%) and only 11 of 372 isolates of other yeast species yielded a false positive result (specificity, 96.8%). Sensitivity and specificity were consistent from one laboratory to another. Using Candida ID medium, the rapid trehalase and maltase test showed a sensitivity of 95% and specificity of 96.2%. Using CHROMagar Candida, sensitivity and specificity were 95.6% and 98.1%, respectively. Using Albicans ID2 medium (tested by two laboratories), the sensitivity was 100% and 98.5% and specificity was 98.1% and 98.2%. In 60% of cases, the test could be performed directly from the primary isolation medium, thus reducing the time for identification.

Conclusion: The rapid trehalase and maltase test was highly reliable for the presumptive identification of *C glabrata* on primary isolation using three different chromogenic media. Direct recognition of *C albicans* by means of their characteristic colour on chromogenic media coupled with one minute trehalase maltase testing performed only on suspect colonies of *C glabrata* allowed for rapid presumptive identification of the two yeast species most commonly encountered in clinical samples.

Candida species are the fourth most commonly encountered nosocomial pathogens, and opportunistic candidiasis has become an important cause of morbidity and mortality in immunocompromised patients.¹ Most emerging yeast pathogens are candida species other than *Candida albicans*, and *Candida glabrata* is now one of the most frequently encountered opportunistic pathogenic yeast species in clinical laboratories.^{2–3} Importantly, some strains show decreased susceptibility to several azole antifungal agents, notably to fluconazole.^{4–5} Thus, early detection and identification of this candida species is essential to start targeted antifungal treatment and to prevent dissemination of infection.

“Opportunistic candidiasis has become an important cause of morbidity and mortality in immunocompromised patients”

To facilitate the identification of yeasts in clinical laboratories, several chromogenic media have been introduced that allow immediate identification of *C albicans*,^{6–8} and may identify other important species such as *Candida tropicalis* and *Candida krusei* by their colony colour.^{9–11} However, no chromogenic medium is yet available that can specifically identify *C glabrata*.

We evaluated a one minute strip trehalase and maltase test, based on the rapid hydrolysis of trehalose by *C glabrata* in conjunction with a maltase test to reduce or eliminate the false positive results from other *Candida* spp. This trehalase and maltase test, using commercially supplied glucose oxidase test strips, has the advantage of requiring both a small inoculum and no incubation time.^{12–13} When evaluated with 482 stock yeast strains grown on different primary plates, this test

showed an overall sensitivity of 95% and a specificity of 98% in a recent study.¹³

The aim of our study was to assess the value of the trehalase and maltase test in routine laboratory practice using freshly isolated yeast strains. For this purpose, four different laboratories using different chromogenic media as primary isolation plates for the culture of *Candida* spp. consecutively applied the trehalase and maltase test on non-*C albicans* colonies.

MATERIALS AND METHODS

Isolates and clinical samples

From September 2001 to September 2002, a total of 944 yeast isolates were prospectively tested in the Medical Parasitology-Mycology Laboratory from Lyon, France (laboratory A), the Parasitology-Mycology Laboratory from St Etienne, France (laboratory B), the Microbiological Laboratory from Newcastle upon Tyne, UK (laboratory C), and the Microbiological Laboratory from Debrousse, Lyon, France (laboratory D). Table 1 reports the number of isolates tested in each laboratory and the respective medium used. The 944 strains comprised 572 *C glabrata* strains and 372 non-*C glabrata* strains; namely: *C parapsilosis* (n = 89), *C albicans* (n = 48), *C tropicalis* (n = 66), *C kefyr* (n = 35), *C krusei* (n = 30), *C inconspicua* (n = 20), *C lusitaniae* (n = 10), *C guilliermondii* (n = 9), *C rugosa* (n = 9), *C famata* (n = 8), *C sphaerica* (n = 8), *C lipolytica* (n = 3), *C holmii* (n = 2), *C norvegensis* (n = 2), *C colliculosa* (n = 1), *C utilis* (n = 1), *C lambica* (n = 1), *S cerevisiae* (n = 20), *Cryptococcus* spp. (n = 3), *Trichosporon asahii* (n = 3), *Trichosporon mucoides* (n = 1), *Zygosaccharomyces* (n = 1), and *Pichia* spp. (n = 2). These 944 strains were isolated from 937 clinical specimens, including bronchoalveolar fluid (n = 224),

Table 1 Results of the trehalase maltase test for the identification of *Candida glabrata* in comparison with the conventional method, obtained in four laboratories with strains isolated on three chromogenic media

Species	Number of isolates yielding a positive trehalase maltase test (number of strains tested)					
	All laboratories		Laboratory A	Laboratory B	Laboratory C	Laboratory D
	All media	Candida ID (bioMérieux)	CHROMagar Candida (Becton Dickinson)	Albicans ID 2 (bioMérieux)	Albicans ID 2 (bioMérieux)	
<i>Candida glabrata</i>	550 (572)	288 (303)	130 (136)	68 (68)	64 (65)	
Other yeasts	361 (372)	8* (210)	1† (53)	1‡ (53)	1‡ (56)	
Sensitivity (95% CI)	96.2%	95.0% (91.8% to 97.1%)	95.6% (90.6% to 98.4%)	100% (94.7% to 100%)	98.5% (91.7% to 100%)	
Specificity (95% CI)	96.8%	96.2% (92.6% to 98.3%)	98.1% (89.9% to 100%)	98.1% (89.9% to 100%)	98.2% (90.4% to 100%)	

* *Candida parapsilosis* (n=1), *C. tropicalis* (n=5), *C. kefyr* (n=1), *C. sphaerica* (n=1); † *C. parapsilosis* (n=1); ‡ *C. tropicalis* (n=1). CI, confidence interval.

stools (n = 163), urine (n = 157), sputum (n = 94), endotracheal aspirates (n = 83), genital (n = 47), blood (n = 42), ear, nose, and throat (n = 41), pus (n = 28), biopsy (n = 16), puncture fluid (n = 16), catheter (n = 13), nail (n = 6), cerebrospinal fluid (n = 2), bile (n = 2), and unknown (n = 3).

Primary isolation media

Depending on the laboratory (table 1) the specimens were streaked on Candida ID (bioMérieux, Marcy l'Etoile, France), CHROMagar Candida (Becton Dickinson, le Pont de Claix, France), or Albicans ID2 (bioMérieux), which were used as primary isolation media. The plates were incubated at 37°C for 24 to 48 hours.

The Candida ID and Albicans ID2 agars are chromogenic media on which *C. albicans* colonies develop a characteristic blue colour. In addition, Candida ID agar contains a second substrate that allows the formation of pink colonies by several other species excluding *C. glabrata*. Thus, on these two media, only the white colonies were tested using the trehalase maltase test to identify potential strains of *C. glabrata*. CHROMagar Candida allows the identification of *C. albicans*, *C. tropicalis*, and *C. krusei* by their colony colour, and on this medium, only the pink colonies needed to be tested using the trehalase maltase test.

Conventional identification

All isolates were identified by conventional methods including germ tube formation, assimilation pattern with ID 32C identification strips (bioMérieux), morphology on rice agar Tween, and latex agglutination with Krusei-Color (Fumouze, Levallois Perret, France).

Reagents

Clinistix reagent strips (Bayer Diagnostics, Puteaux, France), commonly used for semiquantitative measurement of glucose in urine, were used. The strips contained a colorimetric pad impregnated with glucose oxidase and peroxidase, which react with glucose to generate a blue colour. Trehalose and maltose were purchased in the form of 2.5 mg tablets (Rosco, Eurobio Laboratory, Ulis, France). Each tablet was dissolved in 2 ml sterile distilled water to produce solutions of 1.25 g/litre. These reagents were kept for up to one week at 4°C.

Principle of the trehalase maltase test

Briefly, the test is based on the capacity of *C. glabrata* to hydrolyse trehalose (but not maltose) into two glucose molecules in less than one minute. The glucose reacts with the glucose oxidase detection system to give a blue colour. A simultaneous test for maltose, using the same principle as the trehalase test, was systematically applied in parallel to eliminate false positive results yielded by other yeasts. Moreover, because most fungal isolation media contain glucose, a sugar free control strip was used to detect possible carry over of glucose containing agar.

Procedure

Three reagent strips were used for each test. The detection pad of the first strip was hydrated with one drop of trehalose solution, the second strip was hydrated with one drop of maltose solution, and the third with one drop of sterile distilled water. One or two yeast colonies with an identical appearance were picked from the agar plates and smeared directly on to each of the three reagent pads. Care was taken to discard all excess liquid and to sample colonies with care to avoid carry over of agar media containing glucose. A positive test on any strip was indicated by the formation of a blue colouration. Table 2 summarises the interpretation of the test results.

Table 2 Interpretation of the readings

Sugar free control strip	Trehalose strip	Maltose strip	Identification
-	+	-	<i>Candida glabrata</i>
-	+	+	Non- <i>C glabrata</i>
-	-	+	Non- <i>C glabrata</i>
-	-	-	Non- <i>C glabrata</i>
+	+ or -	+ or -	Uninterpretable test

Take home messages

- The rapid trehalase and maltase test is highly effective for the presumptive identification of *Candida glabrata* in the clinical laboratory with strains isolated on one of the three most commonly used chromogenic media—*Candida* ID, CHROMagar *Candida*, or Albicans ID2
- The test is extremely rapid, cost effective, and simple to use
- In addition, the test yields consistent results between different laboratories and offers a useful adjunct to existing methods in routine medical mycology

Statistics

Test sensitivity and specificity were calculated for the identification of *C glabrata* from the strip tests. The 95% confidence intervals of proportions were calculated using Epi info 2002 software.

RESULTS AND DISCUSSION

For the purposes of our study the ID 32 C gallery was regarded as a "gold standard" for *C glabrata* identification. From a total of 944 isolates, (572 *C glabrata* strains and 372 non-*C glabrata* strains) identified by the ID 32 C gallery, the trehalase and maltase test yielded an overall sensitivity and specificity of 96.2% and 96.8%, respectively (table 1). The 11 non-*C glabrata* strains that yielded a false positive result were *C tropicalis* (n = 7), *C parapsilosis* (n = 2), *C kefyr* (n = 1), and *C sphaerica* (n = 1). We tested the performance of the trehalase and maltase test in four laboratories which used three different chromogenic media for primary isolation of yeasts. The sensitivity and specificity results were not significantly different from one medium to another (table 1). Moreover, these results confirm the good interlaboratory reproducibility of the test previously found in two laboratories with stock isolates.¹³

"Direct recognition of *C albicans* coupled with the one minute trehalase and maltase test for suspect colonies of *C glabrata* allowed for rapid presumptive identification of the two yeast species most commonly encountered in clinical samples"

Conventional identification of *C glabrata* is based on morphological features and biochemical tests, such as carbohydrate assimilation, and this often requires up to 48 hours of incubation.^{2,3,6} Because of the low inoculum density required for this rapid test (one to two colonies), the test was done from the primary isolation medium in 60% of cases, reducing the time taken for identification by 24–48 hours. This rapid and cost effective test would be useful for the identification of *C glabrata* in routine practice, enabling an informed decision about the most appropriate antifungal treatment to be made at an earlier stage.

The trehalase and maltase test was of particular use when used in conjunction with chromogenic media. Direct recognition of *C albicans* coupled with the one minute trehalase and

maltase test for suspect colonies of *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 for suspect colonies of *C glabrata*, thus reducing labour time and reagent usage. In a previous study,¹² it was shown that CHROMagar *Candida* was unsuitable for use with the rapid trehalase maltase test. This medium has recently been reformulated by Becton Dickinson,¹⁴ and the results of our study confirm that the rapid trehalase maltase test is now compatible with the use of CHROMagar *Candida*.

In conclusion, our study showed that the rapid trehalase and maltase test is highly effective for the presumptive identification of *C glabrata* in a real clinical setting with strains isolated on one of the three most commonly used chromogenic media—*Candida* ID, CHROMagar *Candida*, or Albicans ID2. This test, which is extremely rapid, cost effective, and simple to use, yields consistent results between different laboratories and offers a useful adjunct to existing methods in routine medical mycology.

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