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CHROMagar Candida as the Sole Primary Medium for Isolation of Yeasts and as a Source Medium for the Rapid-Assimilation-of-Trehalose Test

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The chromogenic medium BBL CHROMagar Candida (CAC) was evaluated as a sole primary medium for the isolation of yeasts from clinical specimens in which yeasts are the primary concern. Additionally, the reliability of the rapid-assimilation-of-trehalose (RAT) test in yielding correct results with isolates taken from CAC was assessed. A total of 270 throat, urine, and genital (TUG) specimens were streaked onto CAC, Sabouraud dextrose agar (SDA), inhibitory mold agar (IMA), and Mycosel (MYC). A total of 69 blood culture broths that were smear positive for yeast were streaked onto CAC and SDA. A 1-h RAT test (NCCLS M35-A) was performed simultaneously on isolates from CAC and SDA. A total of 112 TUG specimens yielded yeast colonies (CAC, 111 colonies; IMA, 105; SDA, 103; MYC, 91). The 69 blood culture yeasts grew on both CAC and SDA. Mixed cultures of yeasts were detected on 11 CAC plates but were unrecognized on other media. Colonies suspected of being *C. glabrata* on 32 CAC plates were all RAT test positive and confirmed to be *C. glabrata*; of 59 colonies with various characteristics of color and morphology on CAC, none were RAT positive, and all were conventionally identified as yeasts other than *C. glabrata* (sensitivity and specificity, 100%). The same isolates from SDA tested for RAT produced six false negatives and no false positives (sensitivity, 81%; specificity, 100%). The results show that CAC can be used as the sole primary medium for recovery of yeasts from clinical specimens. Additionally, isolates grown on CAC yield excellent results with the RAT test utilized in this study.

Candida species are now the fourth-most-common cause of hospital-acquired bloodstream infections (4, 13) and are the organisms commonly sought in fungal cultures of throat, urine, and genital (TUG) specimens. Over the past 4 decades, rates of *Candida* infections have steadily increased, with non-*albicans Candida* species making up a greater proportion of nosocomial infections (4, 13, 14). *Candida glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* constitute the majority of species other than *C. albicans* isolated in most institutions. Of note, *C. glabrata* and *C. krusei* have been observed to be 4- to 32-fold less susceptible than *C. albicans* to fluconazole (3, 11, 12, 14). Rapid species-level identification is therefore an essential task for the clinical mycology laboratory, as it can have a direct bearing on treatment decisions.

The usefulness of a selective and differential medium for the isolation of *Candida* spp. has long been noted (8, 10). CHROMagar Candida (CAC) is a selective medium for the isolation of fungi that simultaneously provides direct differentiation and identification of several *Candida* species (9). The yeasts produce enzymes that react with chromogenic substrates in the CAC medium, producing colonies of different colors. These enzymes are specific, allowing some yeasts to be identified to the species level by their color and colony characteristics. Colonies of *C. albicans* and *C. dubliniensis* appear lighter and darker green, respectively, *C. tropicalis* colonies appear dark blue to metallic blue, and *C. krusei* colonies appear light pink and dry with a light border. Other yeasts are noted to appear cream colored or develop a light- to dark-pink tone.

The manufacturer does not presently claim the ability to identify *C. glabrata* by color or texture, although several studies have stated that, with experience, the species can be identified by colony color and size on CAC (2, 6, 12). In our laboratory, it has been found to be a very subjective call that requires confirmation. Classically, *C. glabrata* identification has been based on growth characteristics and biochemical tests (5) that can take 2 to 3 days to complete. In contrast, a rapid-assimilation-of-trehalose (RAT) test (method of Stockman and Roberts) (1, 7) that requires only 1 h of incubation has been developed specifically for its identification. Because of the increased prevalence of *C. glabrata* in bloodstream and other infections and its decreasing susceptibility to fluconazole, it is imperative that a clinical mycology laboratory be capable of identifying it with certainty as rapidly as possible.

Currently, most clinical laboratories utilize a battery of 2 to 3 primary media for each specimen submitted for fungus culture. For identification to the species level of a yeast other than *C. albicans*, the biochemical testing of the isolate typically takes 1 to 3 days.

The purpose of our study was (i) to evaluate the performance of CAC for use as the sole primary medium for yeasts from selected clinical specimens and (ii) to verify that the RAT test will yield correct results when the isolate is taken from CAC, enabling identification of *C. glabrata* on the same day as colony growth.

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TABLE 1. Yeasts recovered from TUG and blood cultures

Species	No. of isolates recovered from:				
	TUG specimens[a]				Blood cultures[b]
	CAC	IMA	SAB	MYC	
C. albicans	71	64	60	55	24
C. glabrata	25	22	23	16	20
C. parapsilosis	12	9	8	9	10
C. tropicalis	10	7	7	7	8
C. guillermondii	1	1	1	1	1
C. krusei	0	0	0	0	6
C. pelliculosa	1	1	1	1	0
C. dubliniensis	1	1	1	1	0
Rhodotorula sp.	1	0	1	1	0
Total	122	105	103	91	69

[a] A total of 11 mixed cultures were identified on CAC and not on other media.
[b] All blood cultures grew on both CAC and SDA.

TABLE 2. Comparison of the RAT test results for yeast isolates from CAC medium versus those from SDA

Yeast and medium	RAT test result (no. of isolates)	
	Positive	Negative
C. glabrata		
From CAC	32	0
From SDA	26	6
Other *Candida* species[b]		
From CAC	0	59
From SDA	0	59

[a] Isolates from CAC: sensitivity, 100%; specificity, 100%. Isolates from SDA: sensitivity, 81%; specificity, 100%.
[b] See Table 1 for listing of species tested.

MATERIALS AND METHODS

A total of 270 clinical specimens in which yeasts were the anticipated fungus, i.e., TUG specimens, were streaked onto CAC, Sabouraud dextrose agar (SDA), inhibitory mold agar (IMA), and Mycosel (MYC) (all media from Becton Dickinson/BBL, Sparks, Md.). A total of 69 blood cultures yielding a positive signal on a continuous monitoring system (BacT/Alert; bioMerieux, Durham, N.C.) and found to be smear positive for yeast were streaked onto CAC and SDA. The CAC plates were incubated in atmospheric air at 37°C, as recommended by the manufacturer. The other media were incubated at 30°C, the temperature routinely used for fungal cultures in our clinical mycology laboratory. All cultures were read at 48 h and again on days 4 and 7 if no growth was observed on the first reading. If a sparse amount of yeast grew on one agar only, it was eliminated from the study due to likelihood of random sampling. The 1-h RAT test was performed simultaneously on isolates from CAC and SDA for identification of *C. glabrata*. Briefly, 100 μl of RAT broth, prepared in our laboratory (7) and containing yeast nitrogen base, trehalose, bromcresol green, and cycloheximide, was placed into microtiter wells; a heavy inoculum of yeast colonies was emulsified in the broth and incubated uncovered at 37°C in ambient air for 1 h. A positive result was indicated by the blue solution turning yellow to greenish yellow. The solution remaining blue to blue-green indicated a negative result. Confirmatory identification was based on assimilation patterns on API 20C (bioMerieux, Marcy l'Etoile, France) and by microscopic morphology on cornmeal-Tween 80 agar (prepared in lab) (7).

RESULTS

A total of 41% (112 of 270) of TUG cultures and 100% of the smear-positive blood cultures yielded yeasts on at least two inoculated media. From the 112 positive TUG specimens, 122 isolates were detected on CAC, 105 were detected on IMA, 103 were detected on SDA, and 91 were detected on MYC. Eleven mixed cultures were detected only on CAC; mixed characteristics were unrecognized on other media. The mixed cultures consisted of *C. albicans*-*C. glabrata*, *C. albicans*-*C. tropicalis*, and *C. tropicalis*-*C. glabrata.* The species recovered from TUG and blood culture specimens are listed in Table 1. All isolates from both TUG and blood cultures produced the predicted identifiable colors on CAC. *C. albicans* and *C. dubliniensis* colonies appeared light to medium green. These two species were definitively differentiated by ability to grow at 42 to 45°C, by formation of single versus clustered terminal chlamydospores on cornmeal-Tween 80 agar, and by API 20C assimilation patterns. *C. tropicalis* colonies appeared dark blue to metallic blue, and *C. krusei* appeared dry and light pink with a whitish border. *C. glabrata* produced smallish, smooth, light-pink colonies. The appearance of other species ranged from cream to light pink colonies, some resembling the appearance of *C. glabrata.*

A total of 32 colonies suspected of being *C. glabrata* on CAC plates were tested for RAT, and all were positive and simultaneously confirmed to be *C. glabrata*; of 59 colonies with various color and morphology characteristics recovered on CAC, none were RAT positive, and all were further identified as yeasts other than *C. glabrata* (sensitivity and specificity = 100%). The same isolates from SDA tested for RAT produced 6 false negatives and no false positives (sensitivity, 81%; specificity, 100%) (Table 2).

DISCUSSION

More TUG specimens yielded yeast on CAC than on IMA, SDA, or MYC. When yeast was detected on any of the test media, it grew on CAC in all but one case (sensitivity = 99%). The one missed case was probably due to random distribution at the time of inoculation, as only sparse colonies were seen on the other media. Sensitivity values for recovery of yeast on IMA, SDA, and MYC were 96, 93, and 83%, respectively, but no identification to species level or determination of mixed cultures could be directly made on these media. Only CAC was able to detect 11 specimens with mixed cultures of yeasts. The mixed cultures were unrecognized on the other media either because of a low number of the minority species or because of a similarity in colony appearance.

In our study, as in numerous others, *C. albicans*, *C. krusei*, and *C. tropicalis* were readily identifiable on CAC. *C. glabrata* usually produced relatively small, light-pink colonies that with practice could be presumptively differentiated from other non-*albicans Candida* species on CAC at ≤48 h. We found that our 1-h RAT test was 100% sensitive and specific for colonies tested directly from CAC but had a reduced sensitivity of 81% for the same isolates tested from SDA (the precise explanation for this disparity is not known). The combination of growth on CAC and the 1-h RAT test allowed for the identification of *C. glabrata* in as early as 48 h following the initial culture setup.

C. glabrata accounted for 20% of the yeasts isolated from TUG specimens and 29% of the yeasts isolated from blood cultures. When the other species widely acknowledged as identifiable on CAC are included, this medium paired with the 1-h RAT test identified 87 and 84% of the yeasts from TUG and

blood cultures, respectively, on the same day that mature growth occurred. An additional fortuitous benefit is that although the price of one CAC plate was slightly higher than that of any other single medium tested, it was less than that of the combination of any two media that are commonly used in tandem.

Of note is the issue that other brands of CHROMagar and other methods of testing for RAT may not yield the successful results we have found with the combination of products used in this study.

In conclusion, CAC can be used as the sole primary medium for fungus cultures of specimens in which yeasts are the main fungus sought. When compared to other media commonly used, CAC was superior in the primary isolation of yeasts and in identifying mixed cultures of yeasts. The use of BD/BBL CHROMagar Candida with the 1-h RAT test formulation of Stockman and Roberts allows for extremely rapid, same-day identification of colonies of *C. glabrata*. The use of CAC and RAT facilitates increased recovery of yeasts, decreases identification turnaround time, and streamlines the overall workflow in a simple and cost-effective manner.

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