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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Received 1 August 2004/Returned for modification 20 September 2004/Accepted 18 November 2004

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. dublienis 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.
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 yielded a positive signal in a continuous monitoring system (BacT/Alert; bioMerieux, Durham, N.C.) and 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 because of a low number of the minority species or because of mixed cultures that were unrecognized on the other media either because of a low number of the minority species or because of a similarity in colony appearance.

RESULTS

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).

A total of 41% (112 of 270) of TUG cultures and 100% of the smear-positive blood cultures yielded yeast on at least two inoculated media. From the 112 positive TUG specimens, 122 isolates were detected on CAC, 105 were detected on IMA, and 103 were detected on SDA, or MYC. When yeast was detected on any of the test cultures, it grew on CAC in all but one case (sensitivity = 99%).

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.
Stockman and Roberts allows for extremely rapid, same-day CHROMagar Candida with the 1-h RAT test formulation of in identifying mixed cultures of yeasts. The use of BD/BBL CAC was superior in the primary isolation of yeasts and fungus sought. When compared to other media commonly for fungus cultures of specimens in which yeasts are the main 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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