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Evaluation of a Reformulated CHROMagar Candida

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CHROMagar Candida is a differential culture medium for the isolation and presumptive identification of clinically important yeasts. Recently the medium was reformulated by Becton Dickinson. This study was designed to evaluate the performance of the new formula of CHROMagar against the original CHROMagar Candida for recovery, growth, and colony color with stock cultures and with direct plating of clinical specimens. A total of 90 stock yeast isolates representing nine yeast species, including *Candida dubliniensis*, as well as 522 clinical specimens were included in this study. No major differences were noted in growth rate or colony size between the two media for most of the species. However, all 10 *Candida albicans* isolates evaluated consistently gave a lighter shade of green on the new CHROMagar formulation. In contrast, all 26 *C. dubliniensis* isolates gave the same typical dark green color on both media. A total of 173 of the 522 clinical specimens were positive for yeast, with eight yeast species recovered. The recovery rates for each species were equivalent on both media, with no consistent species-associated differences in colony size or color. Although both media were comparable in performance, the lighter green colonies of *C. albicans* isolates on the new CHROMagar made it easier to differentiate between *C. albicans* and *C. dubliniensis* isolates. In conclusion, the newly formulated Becton Dickinson CHROMagar Candida medium is as equally suited as a differential medium for the presumptive identification of yeast species and for the detection of multiple yeast species in clinical specimens as the original CHROMagar Candida medium.

CHROMagar Candida (CR-O) (CHROMagar, Paris, France) is a differential culture medium for the isolation and presumptive identification of clinically important yeasts within 24 to 48 h on the basis of strongly contrasting colony colors (1, 6, 7). The identification of pathogenic yeasts in the laboratory is very important in this era of increasing opportunistic infections and resistant yeasts (2, 5, 8). However, a definitive identification may take several additional days after pure culture. Another advantage of CR-O is that multiple yeast species can be detected in a clinical specimen more easily than on normal mycologic media. Recently, the medium was reformulated by Becton Dickinson (CR-B) (BBL, Cockeysville, Md.) to remove medium components originating from countries with an incidence of bovine spongiform encephalopathy or transmissible spongiform encephalopathy.

This study was designed to evaluate the performance of the new formula of CHROMagar by Becton Dickinson against the original CR-O for recovery, growth, and colony color with stock cultures and with direct plating of clinical specimens. Special emphasis was placed on determining the ability of CR-B to differentiate between *Candida albicans* and the newly characterized species *Candida dubliniensis* (2–5, 8). Both formulations of the CHROMagar Candida medium (CR-B and CR-O) were donated by Becton Dickinson in dehydrated form and were prepared according to the manufacturer's instructions.

A total of 90 yeast isolates from stock cultures representing nine different species and previously identified by the clinical microbiology laboratories at the University of Maryland Hospital were used in this study. Isolates were subcultured twice on Sabouraud dextrose agar medium prior to inoculation of chromogenic media. A single yeast colony was streaked for isolation on both CR-O and CR-B plates and incubated at 37°C without CO_2. Plates were checked for growth after 24 h of incubation and read for visual colony color independently by three investigators after 48 h of incubation. Colony color and morphology were recorded for each isolate in a side-by-side comparison of the two media. Presumptive identifications were made at 48 h.

All 90 yeast isolates tested grew well on both agars after 24 h of incubation at 37°C in ambient air. No major differences were noted in growth rate or colony size between the two media. However, subtle variations in the intensity of colony color were noted between the two media for some of the species.

All 10 *C. albicans* isolates evaluated gave the characteristic light green color with a whitish sheen to the colonies; however, all isolates consistently gave a lighter, yellowish shade of green on CR-B. In contrast, all 26 *C. dubliniensis* isolates, including the type strain CD36 (2), gave the typical dark green color on both media. When different yeast species were mixed in a single broth suspension and simultaneously plated out on CR-B and CR-O, the distinctions in colors and forms were equally easy to recognize on both media (data not shown).

For evaluation of clinical specimens, a total of 522 surveillance stool and respiratory cultures were collected. These included stool and respiratory specimens from oncology patients as well as vaginal and oral specimens from human immunodeficiency virus-positive women. All specimens were directly inoculated onto both media. Plates were incubated at 37°C. All plates were read daily for 10 days and were evaluated for yeast

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TABLE 1. Performance of the reformulated CHROMagar (CR-B) compared to the original CHROMagar (CR-O) with plating of direct clinical specimens

Species	No. of colonies positive with:		No. of colonies with enhanced:			
			Size		Color	
	CR-B	CR-O	CR-B	CR-O	CR-B	CR-O
C. albicans	98	95	41	11	20	10
C. glabrata	48	49	17	5	3	8
C. tropicalis	20	19	6	0	3	1
C. parapsilosis	5	4	1	1	1	1
C. krusei	3	2	3	0	2	0
C. lusitaniae	1	2	1	0	0	1
C. lambica	1	2	1	0	0	0
S. cerevisiae	2	4	1	2	0	2
Total	178	177	71	19	29	23

quantity, color, size, and bacterial contamination. All yeast isolates were identified by using germ tube production, microfermentation, microscopic morphology, cornmeal agar, and API 20C strips (if needed).

A total of 173 specimens were positive for yeast; 158 (91.3%) were positive on CR-B and 157 (90.8%) were positive on CR-O (Table 1). Thirty specimens were positive for multiple species, 25 on both media. Eight species of yeast were recovered, including *C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Saccharomyces cerevisiae, Candida krusei, Candida lusitaniae,* and *Candida lambica*. The recovery rates for each species were equivalent on both media, and bacterial contamination was not a problem on either medium. All germ tube-positive isolates were screened at 45°C, but *C. dubliniensis* was not detected. Color readings were recorded after 48 h of incubation at 37°C as specified in the manufacturer's instructions, and colony colors for all of the clinical yeast species tested were comparable on both media.

The difference in colony color between *C. albicans* and *C. dubliniensis* isolates was, in general, slightly more enhanced on CR-B. Although colony colors were clear and distinguishable after 48 h on both media, colors generally deepened after 72 h of incubation at room temperature. With *C. dubliniensis* especially, the dark green color was found to be more pronounced if plates were incubated for longer than 48 h (e.g., up to 72 h), similar to what has been reported by other investigators regarding the original CHROMagar (6, 8).

With the clinical specimens, colonies on CR-B and CR-O were equal in size at 24 h of incubation. However, color development was inadequate on either medium, and therefore presumptive identification was made after 48 h. At 48 h there was an overall noticeable difference in colony size and enhanced color development. CR-O colony size was generally smaller, and colony color was slightly less developed than that observed on CR-B.

In conclusion, the newly formulated Becton Dickinson CHROMagar Candida medium is as equally suited as a differential medium for the presumptive identification of yeast species and for the detection of multiple yeast species in clinical specimens as the original CHROMagar Candida medium. The subtle variations in color shades observed between the two media when comparing yeast species colony color were insignificant and did not interfere with species identification. The lighter green colonies of *C. albicans* isolates seen with CR-B, however, made it easier to differentiate between *C. albicans* and *C. dubliniensis* isolates on CR-B.

REFERENCES

1. **Beighton, D., R. Ludford, T. Clark, et al.** 1995. Use of CHROMagar Candida medium for isolation of yeasts from dental samples. J. Clin. Microbiol. **33:** 3025–3027.
2. **Jabra-Rizk, M. A., A. A. Baqui, J. I. Kelly, W. A. Falkler, Jr., W. G. Merz, and T. F. Meiller.** 1999. Identification of *Candida dubliniensis* in a prospective study of patients in the United States. J. Clin. Microbiol. **37:**321–326.
3. **Jabra-Rizk, M. A., W. A. Falkler, Jr., W. G. Merz, J. I. Kelley, A. A. M. A. Baqui, and T. F. Meiller.** 1999. Coaggregation of *Candida dubliniensis* with *Fusobacterium nucleatum*. J. Clin. Microbiol. **37:**1464–1468.
4. **Jabra-Rizk, M. A., W. A. Falker, Jr., W. G. Merz, J. I. Kelley, A. A. M. A. Baqui, and T. F. Meiler.** 1999. *Candida dubliniensis* and *Candida albicans* display surface variations consistent with observed intergeneric coaggregation. Rev. Iberoam. Micol. **16:**8–14.
5. **Meiller, T. F., Jabra-Rizk, M. A. Baqui, A. A. M. A. Kelley, J. L. Meeks, W. G. Merz, and W. A. Falker.** 1999. Oral *Candida dubliniensis* as a clinically important species in HIV-seropositive patients in the United States. Oral Surg. Oral Med. Oral Pathol. Radiol. Endod. **88:**573–580.
6. **Odds, F. C., and R. Bernaerts.** 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32:**1923–1929.
7. **Pfaller, M. A., A. Houston, and S. Coffmann.** 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans, Candida tropicalis, Candida krusei*, and *Candida (Torulopsis) glabrata*. J. Clin. Microbiol. **34:**58–61.
8. **Sullivan, D. J., G. Moran, S. Donnelly, S. Gee, E. Pinjon, B. McCartan, D. B. Shanley, and D. C. Coleman.** 1999. *Candida dubliniensis*: an update. Rev. Iberoam. Micol. **16:**72–75.