Turkish J. Pharm. Sci. 3 (1), 41-48, 2006

Short Communication

THE EVALUATION OF CHROMAGAR-*CANDIDA* AS AN ISOLATION MEDIUM FOR IDENTIFICATION OF *CANDIDA* SPECIES ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

Berrin ÖZÇELİK

Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06330 Etiler-Ankara, TURKEY

Abstract

The aim of the study was to evaluate the accuracy of CHROMagar-Candida for the identification of Candida spp. from different clinical specimens. The growth of cultures on plates, containing Sabouraud dextrose agar and CHROMagar-Candida were compared. Non-Candida albicans yeast isolates were further specificated with API 20C (bioMerieux, France). Of the 178 cultures processed, 100% accuracy for C. albicans C. krusei, C. tropicalis and 99% for other Candida spp. were determined. CHROMagar-Candida was found to be a satisfactory isolation medium for different clinical specimens, allowing immediate and correct identification of the commonly encountered yeasts. The use of CHROMagar as a yeast isolation medium in a clinical laboratory for the routine examination appears to be extremely successful in primary isolation and differentiation medium in different sample collections (vaginal specimens, blood cultures, gaita, urine sample, sputum and oropharyngeal swabs) of yeast also it is more rapidly than classical methods.

Key words: *CHROMagar-Candida, Candida spp, identification*

Farklı klinik örneklerden izole edilen Candida türlerinin idendifikasyonu için CHROMAGAR-*CANDIDA*' nın izolasyon besiyeri olarak değerlendirilmesi

Çalışma farklı klinik örneklerden izole edilen Candida türlerinin identifikasyonu için CHROMagar-Candida'nın izolasyon besiyeri olarak doğruluğunun değerlendirilmesi amacıyla yapılmıştır. Kültürlerin Saboraud Dextrose Agar ve CHROMagar-Candida besiyerleri içeren petri plaklarındaki üremesi birbiri ile kıyaslanmıştır. Non-Candida albicans maya izolatları API20C (bioMerieux, France) ile ileri incelemeye alınmıştır. İşleme tabi tutulan 178 kültürde; C. albicans, C. krusei, C. tropicalis için %100 ve diğer Candida spp. için %99 uyum saptanmıştır. Sıklıkla karşılaşılan maya türleri için hızlı ve doğru identifikasyon yapan CHROMagar-Candida, değişik klinik örnekler için uygun bir besiyeri olarak bulunmuştur. CHROMagar'ın klinik mikrobiyoloji laboratuvarlarında maya izolasyon besiyeri olarak rutin tanı amacıyla kullanılması değişik örneklerde (vajinal örnekler, kan kültürü, gaita, idrar, balgam ve orofarengeal sürüntü) mayaların ilk izolasyonu için oldukça başarılı sonuç vermektedir ve aynı zamanda klasik metodlara göre çok daha hızlıdır.

Anahtar kelimeler: *CHROMagar-Candida, Candida türleri, identifikasyon*

∗**Corresponding author**: e-mail: microberr@yahoo.com
e-mail: berrin@gazi.edu.tr

INTRODUCTION

The identification of yeasts by careful observes are often able to be recognized in mixtures of different yeast species when they occur on a single plate according their morphologies or with germ tube test also with assimilation patterns, which were determined with the API.

It is well known that the medium most widely used for the isolation of *Candida* and other yeast species is Sabouraud dextrose agar, however is not a differential medium and colonies of different pathogenic yeast species grown on this agar and cannot be easily distinguished from each other. CHROMagar-*Candida* is a differential medium included chromogenic mix, which is responsible for the differential colors reaction. It is reported by several authors that this medium is capable of the presumptive identification of some clinically important yeast species (1-4).

Propose of this study was to evaluate the accuracy of CHROMagar-*Candida* for the identification of *Candida* spp. from many different clinical samples and with stock cultures. All samples were confirmed by their colony morphologies or with germ tube formation and confirmation was made using API 20C AUX strips.

EXPERIMENTAL

CHROMagar-*Candida was obtained as powdered medium from CHROMagar Company (Paris, France) for this study. Sabouraud Dextrose Agar (SDA)(Oxoid) and Sabouraud Liquid Medium (SLM)(Oxoid) were also used. These mediums were prepared according to the manufactures.*

The yeast like fungi; *Candida albicans* ATCC 10231, *Candida krusei* ATCC 14243, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 13803, *Candida glabrata* ATCC 66032 were obtained from Karadeniz University, Faculty of Medicine Department of Microbiology and Clinical Microbiology and used as control strains. The most common species of identified Candida were *C. albicans* (117), followed by *C. glabrata* (26), *C. parapsilosis* (19), *C. tropicalis* (11) and *C. krusei* (5) respectively. All isolates that obtained from Gazi University Faculty of Medicine Department of Microbiology were recovered from vaginal specimens, blood cultures, gaita, urine sample, and sputum and oropharyngeal swabs. All yeast like fungi were cultured on SDA and passaged at least twice to ensure purity and viability. *Candida* strains were grown at 35^0C overnight while isolated strains were grown at 37^0C for 48 hours. Then culture suspensions were prepared through the guidelines of NCCLS in SLM (5). A total of 178 isolates were screened for their abilities to grow and for their colony colors on CHROMagar-*Candida*. They were identified according to their morphologies on agar and their formation of germ tubes in serum and also confirmed by standard biochemical testing with the API 20C (API bioMerieux, France). Pure colonies were suspended in API 20C inoculation medium (liquid) according to McFarland 0.5 density and were inoculated into the wells which were determined from the firm. They were inoculated at 37^0C for 18-24 hours and after incubation the wells were evaluated according to the change in the colors. The results were obtained with the API20E computer program.

A total of 178 clinical specimens from different samples were inoculated (10μl) in parallel on CHROMagar-*Candida* and Sabouraud dextrose agar to determine how effectively CHROMagar-*Candida* performed as a yeast isolation medium. Colony appearances on CHROMagar-*Candida* were shown in Figure 1 and Figure 2. Colony appearances on CHROMagar-*Candida* were analyzed in terms of sensitivity (number of true positives/number of true positives + number of true negatives) and specificity [number of true negatives/(number of true negatives + number of true positives)] to determine their likely usefulness in the clinical laboratory setting. Results were shown in Table 1.

RESULTS AND DISCUSSION

All isolates were grown on Sabouraud Dextrose Agar and CHROMagar-*Candida*. *Candida albicans*, *Candida tropicalis*, *Candida krusei* developed distinctive colony colors after 48h incubation. *Candida glabrata* and *Candida parapsilosis* developed lighter colony colors than the other species. Table 1 shows that of the 178 cultures 100% accuracy for *C. albicans*, *C.* ***krusei*****,** ***C. tropicalis*** and 99% for other *Candida* spp. The characteristic appearance of *Candida glabrata* and *Candida parapsilosis* was not seen only with the two isolates which prepared from stock cultures.

Candida spp are the fourth common group of nosocomial pathogens isolated from patients on medical, surgical, and intensive care wards (1). The increasing incidence of fungal infections and the reported emergence of resistance to antifungal agents call for the development of differential media and techniques for rapid presumptive identification of yeast and determination of antifungal susceptibility that will enable prediction of outcome in patients suffering from these infections (2, 3). CHROMagar-*Candida* was originally developed for the selective isolation and presumptive identification of some clinically important yeast species such as *C. albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* on the basis of differences in color and surface of colonies (6). CHROMagar-*Candida* is an easy and reliable method for the presumptive identifications of most commonly isolated *Candida* species (1-4). Yucesoy and Marol (3) reported that the sensitivity and specifity rates for CHROMagar-*Candida* were 99.4 100% for *C. albicans,* respectively. They reported the sensitivity of CHROMagar-*Candida* to identify *C. tropicalis*, *C. glabrata*, and *C. krusei* ranged between 90.0 and 100% while the specifity values were found to be 95.4 and 100 for *C. tropicalis* and *C. krusei*, respectively. Beighton *et al.* (4) have reported that CHROMagar-*Candida* is a very useful medium and its use will facilitate the study of yeasts associated with dental diseases. Also in the vaginal swab specimens that have been studied by Houang *et al.* (7), the isolates of *C. albicans*, *C. tropicalis* and *C. krusei* have been correctly identified in the percentages of 100%, 95% and 91% respectively. It is reported by Odds *et al.* (8) that using of CHROMagar-*Candida* was an extremely successful differential detector of yeasts that provides no required germ tube confirmation for *C. albicans*. Likely to this report, Phaller *et al.* (9) have reported that stool or rectal swabs cultures obtained form surgical and neonatal intensive care units do not perform identifications beyond a germ tube test in case of using CHROMagar-*Candida*. Our results are also

in agreement with these prior studies although the isolates were recovered from vaginal specimens, blood cultures, gaita, urine sample, and sputum and oropharyngeal swabs. In agreement with the earlier studies, sensitivity and specificity were determined 100% in our study which was carried on with a larger spectrum of samples of *C. albicans, C. krusei* and *C. tropicalis*. In addition to these studies, Fotedar and al-Hedaithy (10) have reported that CHROMAgar-*Candida* is not only a simple, reliable and cost effective method for the identification of chlamydospore-negative atypical *C. albicans*, but can also be used to differentiate various groups of chlamydospore-negative *C. albicans*. Yera H *et al.* (1) have also reported that this medium is particularly useful for the detection of mixed fungal infections, allowing early and better adapted antifungal treatment.

Table 1. Growth and colony colors of isolates incubated for 48 hours on CHROMagar-*Candida* at 37^0C.

Species **Specificity**	**Total no of isolates**	**Colony colors**	**Sensitivity**
Candida albicans 100	117	Green	100
Candida glabrata 99	26	Light purple	99
Candida parapsilosis 99	19	Light pale pink	99
Candida tropicalis 100	11	Dark blue	100
Candida krusei 100	5	Pale pink	100

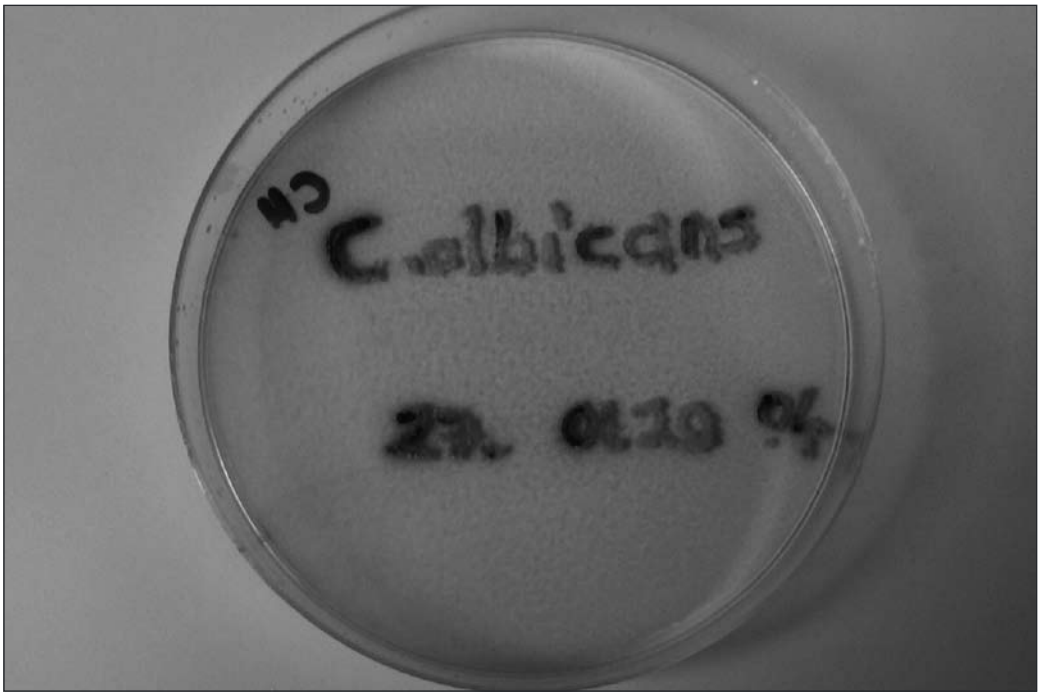

Figure 1. Colonies appearance from suspension of *Candida albicans* incubated for 48 hours at 37^0C on CHROMagar-*Candida*.

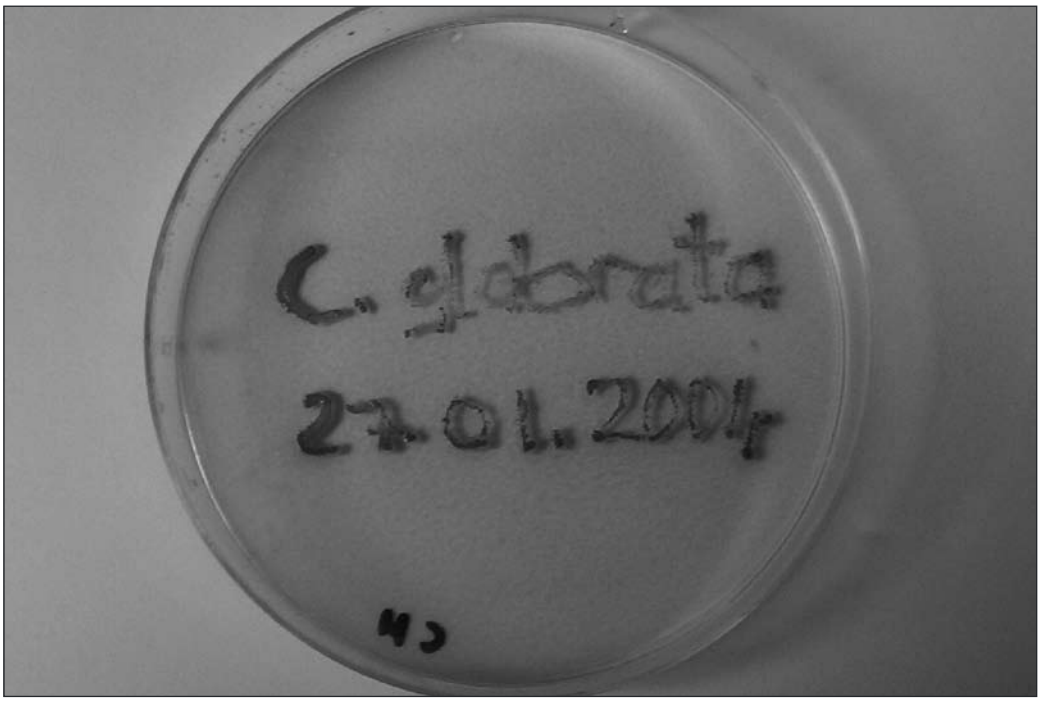

Figure 2. Colonies appearance from suspension of *Candida glabrata* incubated for 48 hours at 37^0C on CHROMagar-*Candida*.

CONCLUSION

The use of *CHROMagar as a yeast isolation medium in a clinical laboratory for the routine examination appears to be extremely successful in primary isolation and differentiation medium. In this study it was determined that identification of yeast from different sample collections such as vaginal specimens, blood* cultures, gaita, urine sample, sputum and oropharyngeal swabs is more rapidly than other classical methods.

In conclusion, it was thought that CHROMagar-*Candida is a satisfactory isolation medium for different clinical specimens, allowing immediate and correct identification of the commonly encountered yeasts.*

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received: 22.12.2005
accepted: 09.02.2006