Journal of Microbiology Research 2012, 2(5): 133-140
DOI: 10.5923/j.microbiology.20120205.04

Strengths and Limitations of different Chromogenic Media for the Identification of *Candida* Species

Ilze Messeir, Pedro M D S Abrantes, Charlene W J Africa*

Medical Microbiology Laboratory, Department of Medical Biosciences, Faculty of Natural Sciences, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa

Abstract The treatment of invasive candidiasis and other *Candida* infections with the appropriate antifungal agent is assisted by the identification of *Candida* isolates to the species level. Rapid and accurate methods of differentiation are therefore imperative if treatment is to be effective, particularly in HIV-positive patients and in pregnant mothers where intervention may be necessary to reduce the risk for preterm delivery. The time used for isolation, identification and detection of mixed cultures may be reduced with the help of available chromogenic media. In this study, five commercial chromogenic media were evaluated for the differentiation of *Candida* species. Six type-strains of *Candida* species were streaked onto each of five different chromogenic media and incubated for up to 4 days at the different temperatures recommended by the manufacturers. This comparative evaluation demonstrated the strengths and weaknesses of each medium employed and found CHROMagar™ Candida and Chromogenic Candida Agar to be the most effective for distinguishing between different *Candida* species.

Keywords *Candida*, Chromogenic Agar, Rapid Species Differentiation

1. Introduction

There has been a significant increase in the number of *Candida* resistant cases in hospital patients in the last 20 years. Predisposing factors include particularly prolonged and increased use of antifungal agents[1] and patients with compromised immune systems, such as HIV-positive patients[2] and pregnant mothers with asymptomatic vaginal candidiasis who run the risk of preterm delivery[3]. Amongst the species most frequently isolated are *Candida albicans* followed by *Candida glabrata*, *Candida tropicalis* and *Candida krusei*[4].

In health, *Candida albicans* is a harmless commensal fungus, while, in immunocompromised patients, it may cause superficial or even life-threatening systemic infections[5]. It is not entirely understood how the mechanisms of change from a non-pathogenic to a pathogenic phenotype occurs. Knowledge of the metabolic activity of *Candida albicans* remains limited even though a great deal of research has been done on aspects of its pathogenicity[5].

Candida dubliniensis is a fairly recently described species of *Candida* with similar characteristics to that of *Candida albicans*. It is clinically important to compare the pathogenesis and management of infection by a newly discovered species, with infection caused by other members of the same genus[6]. *Candida albicans* and *Candida dubliniensis* have the same morphological and physiological characteristics due to the close association in their phylogenetics, e.g. germ-tube and chlamydospore formation[6]. This has caused a problem in differentiating between the two species, with the result that *Candida dubliniensis* strains have been, and will continue to be, identified in the clinical laboratory as *Candida albicans[*6]. To make a precise differentiation between the two species requires PCR-based tests, but due to the high quantities of throughput samples at diagnostic laboratories, this is not feasible and thus PCR-based tests are mostly used in research laboratories[7]. Looking at the phenotypic characteristics is much more inexpensive than that of the genotypic characteristics, and scientists have therefore demonstrated the use of selective and differential media for the presumptive identification of *Candida* species with good sensitivity and specificity[8], thereby reducing the time used for isolation, identification and detection in mixed cultures[9].

The purpose of this study was to perform a comparative evaluation of five different chromogenic media in order to establish which would yield the most reliable differentiation of frequently isolated *Candida* species namely, *Candida albicans*, *Candida dubliniensis*, *Candida tropicalis, Candida krusei* and *Candida glabrata*.

2. Materials and Methods

* Corresponding author:
cafrica@uwc.ac.za (Charlene W J Africa)
Published online at http://journal.sapub.org/ microbiology

2.1. Type-strains of *Candida* Used

A total of six type-strains of *Candida* species were used for the evaluation of the five chromogenic media. Of these type-strains, *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 950), *C. krusei* (ATCC 2159), *C. glabrata* (ATCC 26512) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA.) and *C. albicans* (NCPF 3281) and *C. dubliniensis* (NCPF 3949a) from the National Collection of Pathogenic Fungi (NCPF, Bristol, United Kingdom). These type-strains were stored in frozen stocks in cryovials at -70℃ and cultured twice on Sabouraud's dextrose agar (Oxoid, CM 0041) for 24 hours prior to the inoculation of the chromogenic media.

2.2. Inoculation of Chromogenic Media

Chromogenic media used, included commercially prepared CandiSelect™4 Agar (Bio-Rad, 63746) while, Chromogenic Candida Agar (Oxoid, CM1002A), Bismuth Sulphite Glucose Glycine Yeast agar (BiGGY Agar) (Oxoid, CM0589B) also known as Nickerson's medium[10], modified *Candida* Ident Agar, (Fluka, 94382) and CHROMagar™ Candida (CHROMagar, CA220) were purchased in a dehydrated form and prepared according to the manufacturers' instructions. All plates were left to reach room temperature prior to inoculation if previously stored at -4℃. Type-strains of *Candida* species were inoculated onto the different chromogenic media and each incubated for up to 4 days at the different temperatures recommended by the manufacturers. This was done in triplicate. CandiSelect™4 Agar and CHROMagar™ Candida were incubated at 37℃, modified *Candida* Ident Agar, and Chromogenic Candida Agar were incubated at 30℃, and BiGGY Agar was incubated at 28-30℃. The plates were checked after 24, 48, 72 and 96hrs for growth to determine when (according to the manufacturers' claims) the expected colour, morphology or texture of the colonies appeared, and whether prolonged incubation would affect the results.

2.3. Statistical Analysis

Because of the small sample size no meaningful statistical analyses could be performed.

3. Results

All the type-strains grew on the five different chromogenic media. Some type-strains were more distinguishable than others. The appropriate colour, texture and morphology of the colonies were observed after each 24-hour period for a total of 96 hours and compared with the recommended time period of the manufacturers. Some chromogenic media characterized the different type-strains by colour only while others characterized them by colour, texture and morphology.

Both *C. albicans* type-strains (ATCC 90028 and NCPF 3281) appeared as predicted on CHROMagar™ Candida, modified Candida Ident, and Chromogenic Candida Agar (Table 1). They appeared as pink colonies after 24 hours, which darkened to purple after incubation for 48 hours on CandiSelect™4 Agar. On BiGGY Agar, the predicted colour reactions for *C. albicans* were expressed, while the expected mycelial fringe was not observed even after prolonged incubation of 96 hours. (Table 1)

Table 1. Ability of Chromogenic Media to Accurately Differentiate *Candida albicans* From Other *Candida* Species

Agar	Incubation	Predicted	Observed
CHROMagar™ Candida agar @ 37 ℃	48hrs	green	green-turquoise
Candida Ident Agar, (modified) @ 30 ℃	18-24hrs	light green	light green
Chromogenic Candida Agar @ 30 ℃	24hrs	green	green
CandiSelect™4 Agar @ 37 ℃	24hrs	pink-purple	pink
	48hrs	purple	purple
BiGGY Agar @ 28-30 ℃	48-96hrs	smooth, circular brown-black with slight mycelial fringe	smooth, circular brown colonies. No mycelial fringe even after 96hrs

Colonial morphology of *C.dubliniensis* differed from the predicted patterns for all 5 of the chromogenic media used (Table 2). Although the guidelines for CHROMagar™ Candida, modified Candida Ident Agar and BIGGY Agars predicted that *C.dubliniensis* could not be distinguished, results on the CHROMagar™ Candida revealed that *C. albicans* and *C. dubliniensis* could clearly be distinguished with *C. albicans* colonies yielding a green-turquoise colour while *C. dubliniensis* appeared plain green after 48 hours incubation (Table 2). After a longer incubation period (96 hours), no change was observed in *C. albicans* while colonies of *C. dubliniensis* formed a darker centre, a characteristic clearly distinguishing it from *C. albicans* (Fig. 1a,b).

Chromogenic Candida Agar guidelines predicted a green colour, but we observed translucent light-blue colonies after 24 hours which intensified to dark blue on prolonged incubation of 96 hours (Fig. 1c,d). Prolonged incubation was also required for CandiSelect™4 Agar since the pink-purple colonies predicted after 24 hours only appeared after 72 hours of incubation (Table 2).

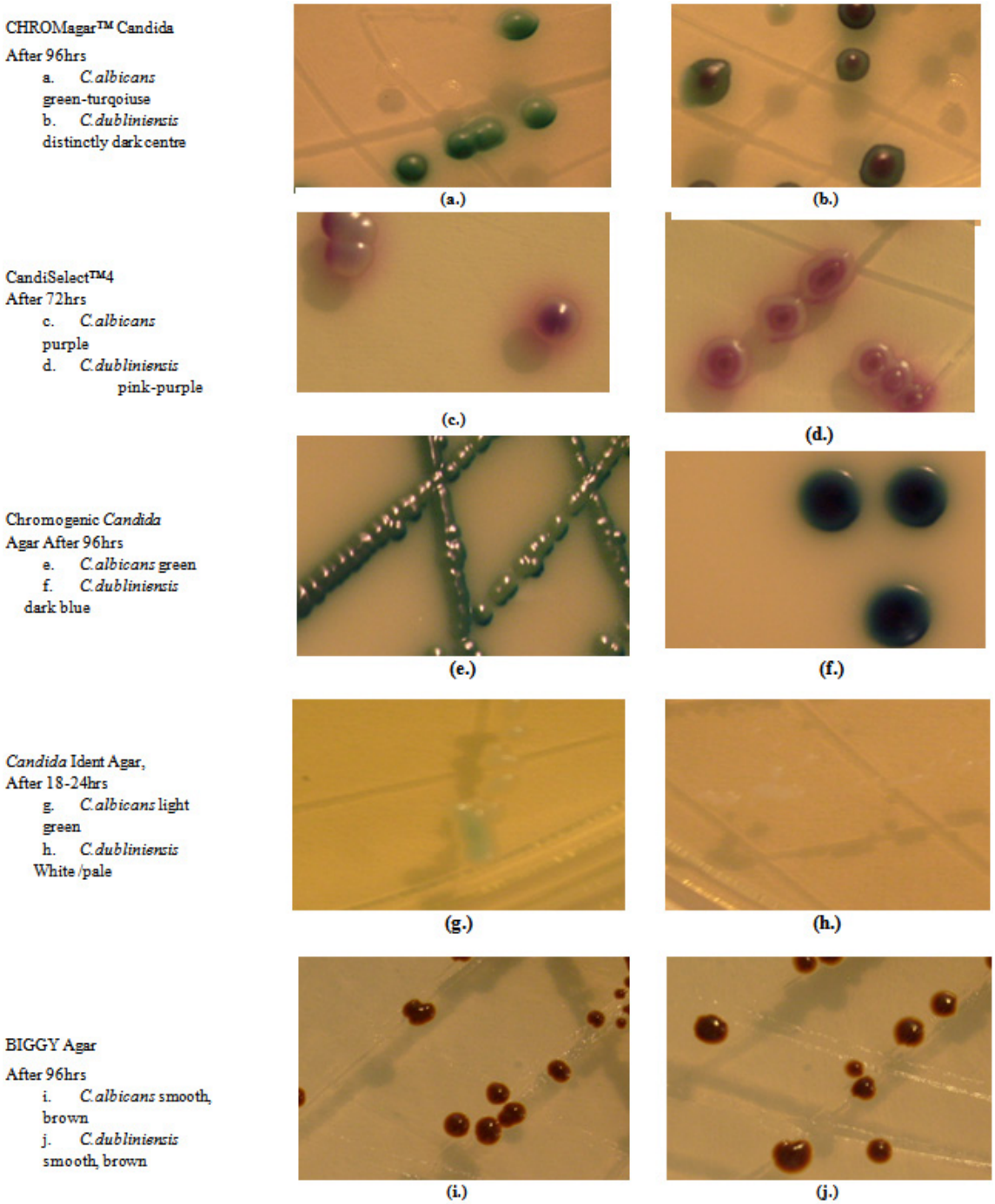

Figure 1. Differentiation of *C.albicans* and *C.dubliniensis* Using Different Chromogenic Media

Table 2. Ability of Chromogenic Media to Accurately Differentiate *Candida dubliniensis* From Other *Candida* Species

Agar	Incubation	Predicted	Observed
CHROMagar™ Candida agar @ 37°C	48hrs	not distinguishable from *C.albicans*	plain green,
	96hrs		green with a dark centre
Candida Ident Agar, (modified) @ 30°C	18-24hrs	ND*	white colonies
	48hrs		metallic green
Chromogenic Candida Agar @ 30°C	24hrs	green	translucent light blue
	25-48hrs		blue
	96hrs		dark-blue
CandiSelect™4 Agar @ 37°C	24-48hrs	Pink to purple	Light pink
	72hrs		pink-purple
BiGGY Agar @ 28-30°C	48hrs	ND*	smooth irregular shaped, light brown
	96hrs		smooth irregular shaped brown

*Not distinguishable from other *Candida* species

Table 3. Ability of Chromogenic Media to Accurately Differentiate *Candida glabrata* From Other *Candida* Species

Agar	Incubation	Predicted	Observed
CHROMagar™ Candida agar @ 37°C	48hrs	not distinguishable (but other species white to mauve)	mauve-colour
Candida Ident Agar, (modified) @ 30°C	18-24hrs	cream white	cream-white to slight pink
Chromogenic Candida Agar @ 30°C	24hrs	variable, natural pigment	beige-cream to light brown
	24-48hrs (max 72hrs)	variable, natural pigment	brown with slight signs of pink
CandiSelect™4 Agar @ 37°C	24hrs	ND*	pale turquoise, flat, shiny, smooth, turquoise center and small white periphery
	48hrs	pale turquoise, flat, shiny, smooth, turquoise center and white periphery	dark turquoise, flat, shiny, smooth, dark turquoise center and white periphery
BiGGY Agar @ 28-30°C	48hrs	not distinguishable	small, cream, opaque

CHROMagar™ Candida, modified Candida Ident Agar, and BIGGY Agar were not able to distinguish *C. glabrata* from other *Candida* species (Table 3), while Candiselect™4 Agar yielded the predicted pale turquoise colonies after 24 hours, which darkened to deep turquoise centred colonies with white peripheries after 48 hours. Chromogenic Candida Agar produced beige-cream to light brown colonies. However, this did not distinguish them from other *Candida* species but when incubated for longer than 72 hours, the colonies started to turn pink.

All of the 5 chromogenic media yielded the predicted results for *C. krusei* (Table 4). Although the guidelines mention silver brown-black, we concede that the reflection of the light in the dark brown colonies could have been interpreted by us as gold rather than silver.

The CHROMagar™ Candida and modified Candida Ident Agar, guidelines predict a metallic blue colony for *C.tropicalis*, but we observed dark-purple blue colonies on the CHROMagar™ Candida (Table 5) and light lilac colonies on modified Candida Ident Agar after 24 hours, which intensified to blue after 48 hours. Neither of the agars grew colonies with a metallic sheen. Growth on CandiSelect™4 Agar appeared to match the overall morphology as described in the guidelines, but the colonies appeared blue and not turquoise in colour. Likewise, the colonies appeared to be similar to the BiGGY Agar guidelines, but no mycelial fringe was evident, nor did the media blacken after 72 hours.

Following the pilot study using only the type-strains, clinical strains from our laboratory collection, previously identified as *C. albicans*, *C. dubliniensis*, *C. krusei*, *C. glabrata* and *C. tropicalis* were also compared for consistency in the evaluation of the chromogenic media. Colony colour and morphology observations from the different clinical strains showed the same results as the type-strains for all chromogenic agars.

Table 4. Ability of Chromogenic Media to Accurately Differentiate *Candida krusei* From Other *Candida* Species

Agar	Incubation	Predicted	Observed
CHROMagar™ Candida agar @ 37°C	48hrs	pink, fuzzy	rough, pink, dry, fuzzy
Candida Ident Agar, (modified) @ 30°C	18-24hrs	purple, fuzzy	light purple, fuzzy
Chromogenic Candida Agar @ 30°C	24-72hrs	brown or pink, dry, irregular	pink with beige to brown periphery, irregular
CandiSelect™4 Agar @ 37°C	24-48hrs	turquoise-blue, rough, dry appearance, irregular	turquoise-blue, rough, dry ,irregular
BiGGY Agar @ 28-30°C	48hrs	large, flat, wrinkled silvery brown-black with brown peripheries; yellow halo diffused into medium	flat, wrinkled, gold glittery dark brown, brown periphery; no halo diffused into medium

Table 5. Ability of Chromogenic Media to Accurately Differentiate *Candida tropicalis* From Other *Candida* Species

Agar	Incubation	Predicted	Observed
CHROMagar™ Candida agar @ 37°C	48hrs	metallic blue	dark purple-blue no metallic appearance
Candida Ident Agar, (modified) @ 30°C	18-24hrs	blue-metallic blue	light lilac
	48hrs		blue
Chromogenic Candida Agar @ 30°C	24-72hrs	blue	blue
CandiSelect™4 Agar @ 37°C	24hrs	ND*	white to light turquoise, mat, uniformly coloured, convex, smooth
	48hrs	intense turquoise, mat, uniformly coloured, convex, smooth	blue, mat, uniformly coloured, convex, smooth
BiGGY Agar @ 28-30°C	48hrs	smooth, dark brown with black centre with mycelial fringe	smooth, dark brown with slightly darker centre, no mycelial fringe
	72hrs	diffuse blackening of media after 72hrs	no diffuse blackening of media, no mycelial fringe

4. Discussion

This study evaluated CHROMagar™ Candida, *Candida* Ident Agar (modified), Chromogenic Candida Agar, CandiSelect™4 Agar and BiGGY Agar for their efficacy in the presumptive identification and differentiation of *Candida* species. An appropriate primary culture medium that assists in the recovery and differentiation of colonies which are phenotypically similar is a vital requirement for the laboratory detection of mixed fungal clinical specimens. Traditional methods for identification of yeast pathogens involves several days and specific mycology media while chromogenic media contains chromogenic substrates which react with enzymes secreted by the organisms to give colour reactions for different species[9] thus complementing traditional methods of identification[11]. CHROMagar™ Candida is the most well-known and widely used chromogenic medium for the identification of different *Candida* species and is the most expensive of the five chromogenic media. Results from mixed cultures are reported to provide results 24 to 48 hours sooner than standard isolation and identification methods. It contains a variety of substrates which interact with the enzymes secreted by the yeast species and has been reported to selectively isolate and identify *Candida* species with a high degree of accuracy[12] sensitivity and specificity[13].

As in our study, previous studies reported green colonies for *C. albicans*[12],[14] dark blue colonies for *C. tropicalis* and pink colonies with a downy appearance for *C.krusei[*8]. Although not clearly distinguishable after 48 hours, prolonged incubation (96 hours) proved useful for differentiating *C.albicans* from *C.dubliniensis*. Modified *Candida* Ident Agar, is a new chromogenic medium on which, we assume, not much research has been done. In this study, modified *Candida* Ident Agar and CHROMagar™ differentiated between the different *Candida* species by colour only. *C. krusei* however, could be differentiated by both colour and texture on both media. With the exception of *C. glabrata*, a more accurate colour expression of the other three species occurred after 48 hours of incubation, which suggested that the colours and texture description of the specific species of *Candida* presented by *Candida* Ident Agar would have been more accurate following an incubation of 48 hours rather than 24 hours. These results confirm that this medium does not reflect the appropriate results suggested by the manufacturer and therefore is not as effective in the differentiation of *Candida* species.

Chromogenic Candida Agar (Oxoid) has been re-named "Oxoid Brilliance Candida Agar" but in this study, we refer to it as "Chromogenic Candida Agar". It is a new commercial ready-to-use chromogenic medium, contains chromogenic substrates which react with the different enzymes of species of *Candida*, such as hexosaminidase and alkaline phosphatase resulting in the expression of a specific colour in the colony. The different colours appear as a result of different enzymes produced by the different species[15]. *C. albicans*, *C. dubliniensis* and *C. tropicalis* produce the enzyme hexosaminidase which results in the colonies being green, but *C. tropicalis* yields dark blue colonies due to other metabolic reactions causing a drop in pH[15]. *C. krusei* yielded brown or pink colonies because it produces alkaline phosphatase and due to a combination of natural pigmentation and some alkaline phosphatase activity, *C. glabrata* yielded a variety of natural colour, such as beige, brown and yellow.

CandiSelect™4 Agar (Bio-Rad) contains two chromogenic substrates which interact with hexosaminidase and phosphatase produced by the different *Candida* species[4], while a combination of antibiotics such as chloramphenicol and gentamicin may suppress bacterial growth. In this study, *C. albicans*, *C. krusei* and *C. glabrata* yielded results described by the manufacturer while the other type-strains did not, thus questioning the reliability of this medium. Each of the different species of *Candida* requires different incubation periods on this medium. Similar results have been reported[4] for *C. krusei*, while identification of *C. tropicalis* and *C. glabrata* were regarded as presumptive only.

In this study, BiGGY Agar was not able to distinguish the different *Candida* species due to the fact that all the type-strains were in the same colour range and that the distinctive characteristics such as the mycelial fringe scarcely occurred and when it did occur, it was never at the recommended incubation period.

We have just touched on the differentiation between *Candida albicans* and *Candida dubliniensis*, since, in addition to looking at the other commonly isolated *Candida* species, we were also interested in establishing whether adj usting incubation times of chromogenic media could adequately differentiate between *Candida albicans* and *Candida dubliniensis*. We believe that we have achieved this.

5. Conclusions

The expression of antifungal susceptibility among different *Candida* species and the misidentification of *C. dubliniensis* as *C. albicans* highlights the potential clinical importance of accurate species differentiation. The use of chromogenic media for the rapid and effective identification of *Candida* species has gained popularity within the clinical laboratory but presents with limitations in that inaccuracies often occur between the reactions described by the manufacturer and the actual results obtained in the laboratory. Differences in colonial morphology may occur as a result of differences in the laboratory conditions under-which the experiments are conducted e.g. the water used for media preparation may be of a different purity, thus affecting the substrate in the medium and thereby producing a different colour expression for specific species. Differences in colour and reflection perceptions by different examiners should also be taken into account. By employing several chromogenic media and optimising the incubation periods for each species,

sometimes deviating from the recommendations of the manufacturers, we were able to establish which media produced the most reliable and consistent results and thus accurately differentiate the *Candida* species commonly infecting HIV-positive individuals and pregnant *Candida*-infected mothers.

This comparative evaluation proved that CHROMagar™ Candida and Chromogenic Candida Agar were the most effective of the chromogenic media evaluated and both yielded the expected colour colonies at the expected time period of incubation as suggested by the manufacturer. Candida Ident Agar (modified) and CandiSelect™4 Agar only yielded results typical of three of the type-strains as suggested by the manufacturer, while BiGGY Agar yielded all of the type-strains in one colour range and none of the differentiating morphological characteristics predicted were ever observed. In order to eliminate inaccuracies in the presumptive identification of *C. dubliniensis*, we strongly support the use of CHROMagar™ Candida since this medium most clearly demonstrated the difference between *Candida albicans* and *Candida dubliniensis* thus reducing error in the identification of the two species.

ACKNOWLEDGEMENTS

This material is based upon work supported financially by the National Research Foundation of South Africa.

Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regards thereto.

REFERENCES

[1] Yun-Liang Yang, Ming-Fang Cheng, Ya-Wen Chang, Tzuu-Guang Young, Hsin Chi, Sai Cheong Lee, Bruno MH Cheung, Fan-Chen Tseng, Tun-Chieh Chen, Yu-Huai Ho, Zhi-Yuan Shi, Chung-Huang H Chan, Ju-Yu Lin, Hsiu-Jung Lo, "Host factors do not influence the colonization or infection by fluconazole resistant Candida species in hospitalised patients", Journal of Negative Results in Biomedicine, vol. 7, pp. 12, 2008.

[2] Heather L Powell, Crystal A Sand, Robert P Rennie, "Evaluation of CHROMagar Candida for presumptive identification of clinically important Candida species", Diagnostic Microbiology and Infectious Disease, vol. 32, no.3, pp. 201-204, 1998.

[3] Christine L Roberts, Kristen Rickard, George Kotsiou, Jonathan M Morris, "Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open label pilot randomised controlled trial", BioMed Central, Pregnancy and Childbirth, vol. 11, pp. 18-23, 2011.

[4] Anne Gaschet, Coralier L'Ollivier, Agnes Laplanche, Odile Vagner, Frederic Dalle, B Cuisenier, S Valot, Alain Bonnin, "Evaluation of CandiSelect4, a new chromogenic medium for isolation and presumptive identification of Candida species from clinical species", Journal de Mycologie Médicale, vol. 18, no.2, pp. 89-95, 2008.

[5] Harald Kusch, Susanne Engelmann, Rüdiger Bode, Dirk Albrecht, Joachim Morschhäuser, Michael Hecker, "A proteomic view of Candida albicans yeast cell metabolism in exponential and stationary growth phases", International Journal of Medical Microbiology, vol. 298, no.3-4, pp. 291-318, 2008.

[6] Mary Ann Jabra-Rizk, Aama Abdullah el Baqui, Jacqueline I Kelley, William A Falkler Jr , William G Merz, Timothy F Meiller, "Identification of Candida dubliniensis in a prospective study of patients in the United States", Journal of Clinical Microbiology, vol. 37, no.2, pp 321-326, 1999.

[7] Oliver Kurzai, Werner J Heinz, Derek J Sullivan, David C Coleman, Matthais Frosch, Fritz A Mühlschlegel, "Rapid PCR test for discriminating between Candida albicans and Candida dubliniensis isolates using primers derived from the pH-Regulated PHR1 and PHR2 genes of C. albicans", Journal of Clinical Microbiology, vol. 37, no.5, pp 1587–1590, 1999.

[8] Véronique Apaire-Marchais, Marie Kempf, Corinne Lefrançois, Agnès Marot, Patricia Licznar, Jane Cottin, Daniel Poulain, Raymond Robert, "Evaluation of an immunomagnetic separation method to capture Candida yeasts cells in blood", BioMed Central Microbiology, vol 8, pp. 157, 2008.

[9] Elena Eraso, María D Moragues, María Villar-Vidal, Ismail H Sahand, Nagore González-Gómez, José Pontón, Guillermo Quindós, "Evaluation of the new chromogenic medium Candida ID 2 for isolation and identification of Candida albicans and other medically important Candida species", Journal of Clinical Microbiology, vol 44, no.9, pp. 3340-3345, 2006.

[10] Duane R Hospenthal, Miriam L Beckius, Karon L Floyd, Lynn L Horvath, Clinton K Murray, "Presumptive identification of Candida species other than C. albicans, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida", Annals of Clinical Microbiology and Antimicrobials, vol 5, pp. 1, 2006.

[11] Carmen Delia Cárdenes, Alfonzo Javier Carrillo-Muñoz, Alfonzo Martinez Arias, Carlos Rodríguez-Alvarez, Alvaro Torres-Lana, A Lopez Sierra, Maria-Pilar Arévalo, "Comparative evaluation of four commercial tests for presumptive identification of Candida albicans", Journal of Microbiological Methods, vol. 59, no.2, pp. 293-297, 2004.

[12] Michael A Pfaller, Alasdair Houston, S Coffmann, "Application of CHROMagar Candida for rapid screening of clinical specimens for Candida albicans, Candida tropicalis, Candida krusei, and Candida (Torulopsis) glabrata", Journal of Clinical Microbiology, vol. 34, no.1, pp. 58–61, 1996.

[13] Venitia M Cooke, RJ Miles, RG Price, G Midgley, W Khamri, AC Richardson, "New chromogenic agar medium for the identification of Candida spp.", Applied and Environmental Microbiology, vol 68, no.7, pp. 3622-3627, 2002.

[14] Mine Yücesoy, Serhat Marol, "Performance of CHROMAGAR Candida and BIGGY agar for identification of yeast species", Annals of Clinical Microbiology and Antimicrobials, vol 2, pp. 8, 2003.

[15] Marie-Thérèse Baixench, Agnes Taillandier, Ann Paugam, "Clinical and experimental evaluation of a new chromogenic medium (OCCA, Oxoid) for direct identification of Candida albicans, C. tropicalis and C. krusei", Mycoses, vol 49, no.4, pp. 311-315, 2006.