Evaluation of Albicans ID₂ and Biggy agar for the isolation and direct identification of vaginal yeast isolates

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In this study, 250 vaginal samples from patients with vulvovaginal candidosis were inoculated onto two chromogenic media, Albicans ID₂ and Biggy agar, as well as onto Sabouraud chloramphenicol agar, yielding a total of 63 yeast (25.2 %) on all three media. These strains were identified as *Candida glabrata* in 20 (31.8 %) samples, *Candida albicans* in 15 samples (23.8 %), *Candida tropicalis* in 10 samples (15.9 %), *Candida krusei* in five samples (7.9 %), *Candida kefyr* in five samples (7.9 %), *Candida dubliniensis* in four samples (6.3 %), *Candida parapsilosis* in two samples (3.2 %) and *Candida guilliermondii* in two samples (3.2 %). Mixed fungal cultures and bacterial growth or filamentous fungi were not detected on any of the selected media. The sensitivity and specificity of the Albicans ID₂ and Biggy agar with regard to the identification of *C. albicans* were 80.0 and 64.6 %, and 86.7 and 56.3 %, respectively. This study showed these two chromogenic media to be as effective as Sabouraud chloramphenicol agar with respect to fungal detection. However, neither Albicans ID₂ nor Biggy agar was sufficient for reliable differentiation of yeasts to the species level.

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INTRODUCTION

Sabouraud dextrose agar was devised by a French dermatologist, Raymond Sabouraud, for the cultivation of dermatophytes in 1894 and is the preferred medium of microbiology laboratories. It is a general-purpose medium that supports the growth of most pathogenic fungi, including *Candida* and other yeast species. However, Sabouraud agar is not a differential medium, and colonies of different yeast species grown on this agar cannot easily be distinguished from each other (Odds, 1991; Odds & Bernaerts, 1994).

Chromogenic media such as Albicans ID₂ and Biggy agar allow rapid and presumptive identification of yeasts on primary plates. An indicator substance in these media allows the differentiation of different species according to their colony colour or morphology (Lipperheide *et al.*, 1993; Silva *et al.*, 2004). Bismuth sulfite glucose glycine yeast or Biggy agar (Oxoid), also known as Nickerson's medium, is a selective and differential medium used in the detection, isolation and presumptive identification of *Candida* species. The bismuth sulfite acts as an inhibitory agent to suppress bacterial growth, which enables the recovery of isolated colonies of *Candida*. *Candida* species produce sulfide and bismuth, which combine to produce brown to black-pigmented colonies and zones of dark precipitate in the medium surrounding colonies of some species through a process of substrate reduction. *Candida albicans*, *Candida tropicalis*, *Candida krusei* and *Candida*

kefyr were reported to be identifiable on Biggy agar by the manufacturer (Nickerson, 1953; Mendel *et al.*, 1960; Kalkanci *et al.*, 1999; Aktas *et al.*, 2001; Yucesoy & Marol, 2003; Silva *et al.*, 2004; Yucesoy *et al.*, 2005).

Albicans ID₂ (bioMérieux) contains a chromogenic substrate for the enzyme hexosaminidase with chloramphenicol and gentamicin. Hydrolysis of this substrate, which is characteristic of *C. albicans*, leads to the appearance of blue-pigmented colonies, whilst non-*albicans Candida* strains remain white (Lipperheide *et al.*, 1993; Rousselle *et al.*, 1994; De Champs *et al.*, 1995; Baumgartner *et al.*, 1996; Contreras *et al.*, 1996; Freydière *et al.*, 1997; Hoppe & Frey, 1999; Ilkit *et al.*, 1999; Fricker-Hidalgo *et al.*, 2001; Godoy *et al.*, 2001; Cárdenes *et al.*, 2002, 2004; Yucesoy *et al.*, 2005). Albicans ID₂ has been improved and the new plates are known as Candida ID₂. They have two chromogenic substrates and give blue colonies for *C. albicans*, pink colonies for certain non-*albicans Candida* species such as *C. tropicalis*, *Candida lusitanae* and *C. kefyr*, and white for the rest.

The aim of this study was to compare the performance of the chromogenic media Albicans ID₂ and Biggy agar with that of a reference medium, Sabouraud chloramphenicol agar (SCA), for rapid and non-microscopic presumptive identification of yeast species obtained directly from clinical specimens, as well as to discuss the efficacy of chromogenic media in the mycology laboratory by using our data from 1998.

METHODS

Study population. A total of 250 patients with complaints of vaginal discharge, dyspareunia, burning and itching diagnosed as vulvovaginal candidosis were included in the study in 1998. However, patients with pregnancy, diabetes mellitus, immunodeficiency, a history of corticosteroid, antibacterial or antifungal drugs used in the past month, or infected with *Trichomonas* spp. or with bacterial vaginosis were excluded from the study.

Procedure. Only one sample was examined from each patient. Vaginal samples were examined in order to determine the effectiveness of Albicans ID₂ and Biggy agar for the growth and identification of *Candida* species in comparison with the routinely used SCA. Samples were taken with sterile swabs using a dry sterile speculum and inoculated into a Sabouraud 2% glucose broth with 1 ml liquid glucose for their transport to the laboratory and kept in this broth for a maximum of 30 min before inoculation of plates by streaking the swab.

Commercial media. Biggy agar and Sabouraud agar (Difco Laboratories) were purchased as powdered media and prepared according to the manufacturer's instructions in Petri dishes containing 15 ml of the liquid medium. Chloramphenicol (100 µg ml⁻¹) was added to the Sabouraud agar and the media were stored at 4 °C until use. Albicans ID₂ is a commercial ready-to-use medium.

Evaluation of the media. All specimens were inoculated onto Albicans ID₂, Biggy agar and SCA in parallel. After inoculation, the Albicans ID₂ and SCA cultures were incubated at 37 °C, whilst Biggy plates were incubated at 30 °C, according to the manufacturers' instructions. Interpretation of the results was carried out after 24 and 48 h of incubation.

Identification. All yeast isolates observed on Albicans ID₂ and Biggy agar were identified by colony pigmentation and morphology according to the manufacturers' instructions. The criteria for the identification of *C. albicans* on the two chromogenic media were any blue colour (including weak blue) on Albicans ID₂ and a dark brown-black colour with a yellow zone on Biggy agar. All colonies on SCA were identified by conventional methods, i.e. germ-tube production in human serum at 37 °C for 2 h, micromorphology on cornmeal Tween 80 agar according to the Dalmau method and the commercial API 20C AUX kit (bioMérieux) (Buckley, 1989). *Candida dubliniensis* was differentiated from *C. albicans* by abundant chlamydospore formation on cornmeal Tween 80 agar, the absence of fermentation of xylose and methyl α-D-glucoside on API 20C AUX and the absence of

growth at 45 °C. The reference strains *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 22019 were used as quality controls.

Statistical analysis. The sensitivity and specificity of the colony appearances of *C. albicans* on Albicans ID₂ and Biggy agar were determined as the number of true positives/(number of true positives + number of false negatives) and the number of true negatives/(number of true negatives + number of false positives), respectively.

RESULTS

A total of 250 vaginal samples were cultured on Albicans ID₂, Biggy agar and SCA. In 187 samples (74.8%), no growth was observed, while 63 samples (25.2%) yielded yeast fungi on all three media. These strains were identified as *Candida glabrata* in 20 samples (31.8%), *C. albicans* in 15 samples (23.8%), *C. tropicalis* in 10 samples (15.9%), *C. krusei* in five samples (7.9%), *C. kefyr* in five samples (7.9%), *C. dubliniensis* in four samples (6.3%), *Candida parapsilosis* in two samples (3.2%) and *Candida guilliermondii* in two samples (3.2%) (Table 1). The results were the same after 24 and 48 h of incubation for both media. Mixed fungal cultures and bacterial growth or filamentous fungi were not detected on either of the chromogenic media or SCA.

Twelve (80%) of the 15 *C. albicans* strains produced blue colonies and three (20%) of the strains produced white colonies on Albicans ID₂ medium, whilst seven (14.6%) of the 48 non-*albicans* *Candida* strains, namely four *C. dubliniensis*, two *C. tropicalis* and one *C. glabrata*, produced blue colonies, 10 (20.8%) produced weak-blue colonies and 31 (64.6%) produced white colonies.

On Biggy agar, *C. kefyr* colonies were easily distinguished from those of the other species by their reddish-brown colour, medium size, straight surfaces and projected edges. *C. albicans* and *C. dubliniensis* colonies were distinguished from the other species by the formation of dark brown-black colonies and small mycelial fringes, but it was not possible

Table 1. Detection and colour of yeast fungi on Albicans ID₂ and Biggy agar

Species	Albicans ID ₂			Biggy agar			
	Blue	Weak-blue	White	Reddish brown	Dark-brown with yellow zone	Brown with yellow zone	Light-brown with yellow zone
<i>C. glabrata</i> (20)	1	4	15	—	—	10	10
<i>C. albicans</i> (15)	12	—	3	—	13	1	1
<i>C. tropicalis</i> (10)	2	5	3	—	7	3	—
<i>C. krusei</i> (5)	—	1	4	—	3	1	1
<i>C. kefyr</i> (5)	—	—	5	5	—	—	—
<i>C. dubliniensis</i> (4)	4	—	—	—	4	—	—
<i>C. parapsilosis</i> (2)	—	—	2	—	2	—	—
<i>C. guilliermondii</i> (2)	—	—	2	—	—	2	—
Total (63)	19	10	34	5	29	17	12

to differentiate them from each other. *C. tropicalis*, *C. krusei* and *C. parapsilosis* formed dark-brown colonies with slight mycelial fringes, but it was difficult to distinguish them from each other, as well as from the other species. However, whilst 10 of the 20 *C. glabrata* colonies formed brown colonies with yellow zones, 10 formed light-brown colonies with yellow zones, in addition to the two *C. guilliermondii* strains with brown colonies surrounded by yellow zones, and hence they could not be distinguished from each other. The sensitivity and specificity of Albicans ID₂ and Biggy agar with regard to the identification of *C. albicans* were 80.0 and 64.6 %, and 86.7 % and 56.3 %, respectively.

DISCUSSION

In the literature, it has been noted that Biggy agar together with Pagano–Levin agar and Nickerson's medium with malt extract medium present similar results for the isolation of yeast fungi from vaginal samples (Mendel *et al.*, 1960; Becker & Schweisfurth, 1971). Silva *et al.* (2004) did not observe significant differences among SCA, Biggy agar and CHROMagar Candida in the detection of yeast fungi in different samples, with a lower isolation rate on Pagano–Levin agar. The authors also reported that more than one culture medium should be used for adequate primary isolation. In another study, comparing the isolation of species on SCA (100 %) with that on Candida ID agar (95.2 %) and Albicans ID₂ (91.2 %), it was found that the SCA allowed better isolation in different clinical samples compared with the other two media (Fricker-Hidalgo *et al.*, 2001). Although the production of Albicans ID₂ has been stopped, this paper addresses the efficacy of chromogenic media for primary isolation and identification of yeast fungi from vaginal samples. In our study, evaluation of three different culture media yielded the same results in terms of yeast isolation.

For the rapid identification of *C. albicans*, the sensitivity and specificity of Albicans ID₂ have been estimated as 92.7–100 and 86.6–100 %, respectively (Lipperheide *et al.*, 1993; Rousselle *et al.*, 1994; De Champs *et al.*, 1995; Baumgartner *et al.*, 1996; Contreras *et al.*, 1996; Freydière *et al.*, 1997; Hoppe & Frey, 1999; Ilkit *et al.*, 1999; Fricker-Hidalgo *et al.*, 2001; Godoy *et al.*, 2001; Cárdenes *et al.*, 2002, 2004; Yucesoy *et al.*, 2005). In our study, the sensitivity of Albicans ID₂ medium was 80.0 % and the specificity was 64.6 %, much lower than the results of the studies mentioned above. This may be due to the lower isolation rate in the present study. On the other hand, a surprising result obtained in our study was the isolation of a panel of *Candida* species with *C. glabrata* as the primary species. More recently, Niemann *et al.* (2005) reported the detection of *Candida* species in 173 out of 600 vaginal samples (28.8 %), with 85.5 % of them being *C. albicans*, 8.7 % *C. glabrata*, 1.2 % *C. krusei*, 0.6 % *C. dubliniensis* and 4 % other *Candida* species. The authors had isolated *C. dubliniensis* only once, from a non-symptomatic, HIV-seronegative pregnant proband.

Recently, it was reported that Albicans ID₂ medium did not enable differentiation between *C. albicans* and *C. dubliniensis*, correlating with our study (Fricker-Hidalgo *et al.*, 2001; Godoy *et al.*, 2001). In contrast, Cárdenes *et al.* (2002) noted that they managed to distinguish all 38 *C. dubliniensis* strains on Albicans ID₂ medium. In other studies, *C. tropicalis* was the main source of false-positive identification on this medium (Lipperheide *et al.*, 1993; Rousselle *et al.*, 1994; De Champs *et al.*, 1995; Baumgartner *et al.*, 1996; Hoppe & Frey, 1999; Ilkit *et al.*, 1999; Fricker-Hidalgo *et al.*, 2001; Godoy *et al.*, 2001; Cárdenes *et al.*, 2002, 2004). In accordance with these findings, in 10 *C. tropicalis* strains we observed two producing blue colonies and five producing weak-blue colonies as a false-positive result, whilst only three strains produced white colonies as a true-positive result. In addition, five *C. glabrata* and one *C. krusei* produced blue or weak-blue colonies and could not be distinguished from *C. albicans* (Table 1).

Mendel *et al.* (1960) reported that bacterial inhibition by Biggy agar was superior to that of Pagano–Levin agar in the identification of vaginal samples. Kalkanci *et al.* (1999) determined that *C. kefyr* and *Candida rugosa* strains were easily distinguished from other species, but the colonies produced by such species had no features that could be used for identification on Biggy agar. In a study by Aktas *et al.* (2001), it was stated that *C. albicans*, *C. kefyr* and *C. glabrata* colonies were easily identified because of their different characteristics.

Yucesoy & Marol (2003) reported that it was difficult to differentiate the light or light and/or dark-brown colours of *C. albicans* and *C. tropicalis*, respectively. In their study, only two species showed a typical, distinctive appearance on Biggy agar. One of these was *C. krusei*, which produced typical large, rough, dark-brown colonies with a surrounding yellow zone, whilst the other was *C. parapsilosis*, which formed light brown–greenish, grey, cream-coloured colonies. In another study reported by Yucesoy *et al.* (2005), the sensitivity and specificity of CHROMagar Candida, Biggy agar and Albicans ID₂ agar for the detection of *C. albicans* were 100 and 100 %, 91 and 92.7 %, and 99.2 and 92.7 %, respectively. The authors also reported that few isolates of *C. parapsilosis* and *C. kefyr* and all isolates of *C. glabrata* grew weakly on Biggy agar. The lower sensitivity and specificity of Biggy agar for the identification of commonly isolated *Candida* species potentially limits the clinical usefulness of this agar (Kalkanci *et al.*, 1999; Aktas *et al.*, 2001; Yucesoy & Marol, 2003; Yucesoy *et al.*, 2005). Our results were found to be consistent with only two of the above-mentioned reports in terms of *C. kefyr* differentiation from other species; however, we could not identify features of other species (Kalkanci *et al.*, 1999; Aktas *et al.*, 2001).

This study reconfirms that both Albicans ID₂ and Biggy agar are effective media for the isolation of yeast fungi. However, these two media could fail to identify the correct *Candida* species, e.g. three isolates of *C. albicans* produced

white colonies on Albicans ID₂ agar. In addition, in some cases, several *Candida* species could not be distinguished from each other on Biggy agar. In conclusion, the chromogenic media are not sufficient for reliable differentiation of yeasts to the species level and additional classical differentiation methods, e.g. subcultivation on rice agar, fermentation and assimilation techniques (e.g. ID 32C), are necessary to identify clinical yeast isolates.

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