Prospective evaluation of the new chromogenic medium CandiSelect 4 for differentiation and presumptive identification of the major pathogenic *Candida* species

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The rapid identification of pathogenic yeasts is a crucial step in ensuring that effective antifungal treatment is started as early as possible. CandiSelect 4 (CS4; Bio-Rad) is a new chromogenic medium for the isolation of fungi, the direct identification of *Candida albicans* and the presumptive identification of the major pathogenic *Candida* species. The performance of CS4 was compared with that of another chromogenic medium, CHROMagar *Candida* (CA; Becton Dickinson). For primary cultures, 502 of the 1549 (32 %) samples were culture-positive. A total of 542 yeasts were isolated including 465 monomicrobial and 37 mixed cultures: 392 *C. albicans*, 60 *Candida glabrata*, 25 *Candida tropicalis*, 12 *Candida krusei* and 53 other *Candida* species. The percentage of *C. albicans* isolates that could be identified directly after 24, 48 and 72 h culture was 31.6, 82.9 and 92.1 %, respectively, for CS4, and 32.9, 82.9 and 91.1 % for CA. The presumptive identification of *C. glabrata*, *C. tropicalis* and *C. krusei* was evaluated after 48 h incubation. The percentage of strains with morphologically typical colonies was 80, 68 and 84.6 %, respectively, for CS4 compared with 75, 76 and 76.9 % for CA. For pure subcultures, from 24 h, all isolates of *C. albicans* ($n=21$) were directly identifiable on the two chromogenic media CA and CS4. At 48 h, the proportion of typical strains observed on the two chromogenic media was identical for *C. glabrata* (85 %) and *C. krusei* (100 %). A slight difference in favour of CS4 was observed for *C. tropicalis* (100 vs 95 %). CS4 also allowed the growth of several other fungi. CS4 can be recommended as a primary isolation medium for the identification of *C. albicans*, and for the rapid and effective differentiation of the major pathogenic *Candida* species.

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INTRODUCTION

The incidence of invasive fungal infections has increased steadily over the past two decades (Eggimann *et al.*, 2003; Marr, 2004; Richardson, 2005). With recent advances in medical and surgical intervention and the increasing population of immunocompromised patients, the diversity and list of human fungal pathogens continue to grow (Walsh *et al.*, 2004). It is widely accepted that *Candida* species are a common cause of nosocomial infection with significant associated morbidity, mortality and increased health-care costs (Eggimann *et al.*, 2003; Nucci & Marr,

2005; Pasqualotto *et al.*, 2005; Richardson, 2005). A recent SCOPE study indicated that *Candida* is the fourth most-frequent cause of nosocomial bloodstream infection accounting for 9 % of bloodstream infections overall (Wisplinghoff *et al.*, 2004). *Candida albicans* is the most common *Candida* species implicated in these infections, but non-*albicans* species such as *Candida glabrata* and *Candida krusei* are increasing in frequency. Furthermore, infections caused by these latter species often vary in their antifungal susceptibility (Sanglard & Odds, 2002). According to most published guidelines for the treatment of candidiasis, rapid and accurate diagnosis of infection is required before appropriate treatment can be instigated (Buchner *et al.*, 2002; Pappas *et al.*, 2004; Rex *et al.*, 2000). Several

Abbreviations: CA, CHROMagar *Candida*; CS4, CandiSelect 4.

chromogenic media have been developed to help in the rapid identification of yeasts (Cardenes *et al.*, 2002; Cooke *et al.*, 2002; Fricker-Hidalgo *et al.*, 2001; Letscher-Bru *et al.*, 2002; Odds & Bernaerts, 1994; Rousselle *et al.*, 1994). These media contain chromogenic substrates that react with enzymes secreted by yeast cells, resulting in colonies with various pigmentations. These enzymes are species-specific, allowing organisms to be identified to species level by their colour and colony characteristics. The first generation of these media was designed for the isolation and identification of *C. albicans* in a single step according to the specific pigmentation of yeast colonies. The use of such media also makes it easier to detect mixed yeast cultures (Cardenes *et al.*, 2002; Letscher-Bru *et al.*, 2002; Odds & Bernaerts, 1994). CandiSelect 4 (CS4) chromogenic medium (Bio-Rad) has recently been developed for the identification of *C. albicans* and the presumptive identification of the major pathogenic yeast species (*C. glabrata*, *Candida tropicalis* and *C. krusei*). This prospective study evaluated the performance of CS4 in comparison with that of CHROMagar *Candida* (CA), another chromogenic medium used widely in the clinical microbiology laboratory. This study was designed to evaluate CS4 as a primary isolation medium to identify yeasts directly from clinical specimens.

METHODS

Three different studies were conducted to evaluate the performance of CS4 medium. (i) One thousand five hundred and forty-nine clinical specimens [474 urine, 319 tracheal, 173 oral, 129 anal, 122 sputa, 114 bronchial aspirates, 71 vaginal, 43 stools, 30 ear, nose and throat, 27 bronchoalveolar lavage and 47 miscellaneous (e.g. catheter, drains, ascites)] were plated onto CS4 and CA in a prospective study conducted between February and July 2004. Each non-fluid specimen was suspended in sterile 0.85% physiological saline and 0.02 ml of this suspension was then streaked onto plates of medium. Both media were used for primary isolation in parallel with Sabouraud's dextrose agar (SDA). (ii) Eighty-eight strains, originally isolated from clinical specimens in the bacteriology laboratory and transferred to the mycology laboratory for species identification, were subcultured onto CS4 and CA media. These strains were isolated from 85 samples [24 urine, 14 tracheal, 13 vaginal swabs, 11 stools, eight bronchial aspirates or bronchoalveolar lavage, five sputa, four oral and six miscellaneous (e.g. blood cultures, peritoneal)]. Eighteen strains from our reference collection were also tested (stock isolates). (iii) The growth of less common yeasts and filamentous fungi was also tested using strains from our stock collection.

Plates were incubated at 37 °C for 24–48 h; if no growth was observed, incubation was extended up to 72 h. If an infection with *Cryptococcus neoformans* was suspected, a second plate was inoculated in parallel and incubated at 30 °C. Each plate was read with emphasis placed on recording the colony colour, the number, size and texture of colonies, and the presence of colour diffusion into the surrounding agar.

The reference procedures for identifying yeast species comprised germ tube production, micro-fermentation, microscopic morphology, chlamydosporulation on rice-agar-Tween medium, rapid identification tests (Bichrolatex *albicans*, GLABRATA RTT and Krusei Color; Fumouze Laboratory) and API 32C assimilation tests, if required (bioMérieux).

Statistical analysis was performed using SPSS 11.0 software, and McNemar's test (for paired observations) was used for analysis of fungal c.f.u. (CS4 vs CA) at 24, 48 and 72 h. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

With these two chromogenic tests, the most specific morphology is seen with 48 h colonies. Typically on CS4, colonies produced by *C. albicans* are pink to purple, with a purple pigmentation which diffuses out around the colonies. Intense turquoise pigmented colonies, with a mat, uniformly coloured, convex, smooth morphotype, are indicative of *C. tropicalis*. Light-turquoise colonies (white periphery and turquoise centre), with a flat, shiny, smooth morphotype, are suggestive of *C. glabrata*. Turquoise-blue colonies, with a characteristically rough morphotype, a dry appearance and an irregular outline, are typical of *C. krusei* (Fig. 1). Following incubation for 48 h, colonies that remained white or pale-blue in colour were identified by conventional methods. A total of 1549 clinical samples (primary cultures) and 106 stock strains (subcultures) were analysed.

For pure subcultures, among the 106 isolates, 21 were identified as *C. albicans*, 33 as *C. glabrata*, 21 as *C. tropicalis* and five as *C. krusei*. The other isolates were *Candida parapsilosis* ($n=8$), *Candida kefyr* ($n=7$), *Candida lusitanae* ($n=3$), *Candida* sp. ($n=3$) and *Saccharomyces cerevisiae* ($n=5$). From 24 h, all strains of *C. albicans* were directly identifiable on the two chromogenic media CA and CS4. At 48 h, the proportion of typical strains observed on the two chromogenic media was identical for *C. glabrata* (85%) and *C. krusei* (100%). A slight difference in favour of CS4 was observed for *C. tropicalis* (100 vs 95%).

For primary cultures, among the 1549 specimens, 502 (32.4%) were culture-positive for one or more yeast species. Thirty-seven samples (7.4%) contained two ($n=34$) or three ($n=3$) yeast species in combination and the other 465 specimens were monomicrobial. A total of 542 yeast isolates were recovered on at least one of the three media and identified by conventional methods. The distribution of the isolated yeasts or yeast-like species is summarized in Table 1. The sensitivity of direct identification of *C. albicans* in mono- and polymicrobial samples using CS4 and CA media is shown in Table 2. After 72 h incubation at 37 °C, the sensitivity of the two media was 92.1 and 91.1%, respectively, with no false-positive results. The presumptive identification of other species is shown in Table 3. CS4 was superior to CA for *C. glabrata* (80 vs 75%) and *C. krusei* (92 vs 83%), but less effective for *C. tropicalis* (68 vs 76%). Statistical analysis of these data did not demonstrate a significant difference between CS4 and CA. Concerning the extent of fungal growth (for all species), there was no significant difference between CS4 and CA at 48 h, but CS4 was significantly better after 72 h ($P=0.0083$).

When the data obtained for *C. albicans* are considered alone, the difference in growth density was in favour of CS4 after

Fig. 1. Appearance of *Candida* species isolated directly from monomicrobial or polymicrobial clinical specimens after 48 h incubation at 37 °C. Colonies produced by *C. albicans* (a, c) are pink to purple, with a purple pigmentation which diffuses out around the colonies. Intense turquoise pigmented colonies, with a mat, uniformly coloured, convex, smooth morphotype, are indicative of *C. tropicalis* (b, c). Light-turquoise colonies (white periphery and turquoise centre), with a flat, shiny, smooth morphotype, are suggestive of *C. glabrata* (c). Turquoise-blue colonies, with a characteristically rough morphotype, a dry appearance and an irregular outline, are typical of *C. krusei* (d).

48 h and 72 h incubation ($P=0.04$ and $P=0.02$, respectively). This was particularly obvious for specimens with an initial low fungal load. Thus after 72 h incubation, 346 strains of *C. albicans* were isolated on CS4 compared with only 331 on CA; the proportion of samples that were negative was therefore 5.5 and 9.6 %, respectively. Other *Candida* species producing creamy colonies that are white or pale-blue on CS4 were not distinguishable to the species level with this chromogenic medium. The isolates tested (either as subcultures or primary cultures) that fell into this group included *C. parapsilosis* ($n=32$), *C. kefyr* ($n=23$), *C. lusitaniae* ($n=5$), *Candida norvegensis* ($n=2$) and *Candida guilliermondii* ($n=1$). The selectivity of CS4 was determined with several polymicrobial specimens; only one bacterial isolate, *Klebsiella pneumoniae*, was recovered from broncho-alveolar lavage.

CS4 also allowed the growth of *Cryptococcus neoformans*, *Geotrichum* spp., *Trichosporon* spp., *Scedosporium*

apiospermum, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Mucor* spp. and *Rhizopus* spp.

It should be noted that the majority of isolates that did not develop the expected colour on CS4 (Table 3) were recovered from tracheal-bronchial specimens, mucous expectorations or stool samples. In addition, some of these specimens stained the agar blue at the point at which they were deposited; it is therefore recommended that these types of specimen should be streaked at closely spaced intervals to obtain well-isolated colonies away from the deposit. Concerning the specificity of colony colour, no false-positive reaction was observed for *C. albicans*. Phosphatase activity is particularly intense in *C. tropicalis*; however, some strains of *C. parapsilosis*, *C. kefyr*, *C. guilliermondii* and *C. lusitaniae* which have more intense phosphatase activity can give pale-turquoise colonies after 48 h incubation. Some strains of *C. kefyr* and *C. parapsilosis*

Table 1. Yeast species isolated from 502 mono- and polymicrobial clinical specimens after 72 h incubation at 37 °C on chromogenic media

Nature of samples (no.)	Isolated yeast and yeast-like species	No. of specimens	No. of yeast strains detected on any media
Monomicrobial (<i>n</i> =465)	<i>C. albicans</i>	366	366
	<i>C. glabrata</i>	38	38
	<i>C. tropicalis</i>	18	18
	<i>C. krusei</i>	7	7
	<i>C. kefir</i>	11	11
	<i>C. parapsilosis</i>	18	18
	<i>C. guilliermondii</i>	1	1
	<i>S. cerevisiae</i>	2	2
	<i>C. lusitaniae</i>	2	2
	<i>Geotrichum capitatum</i>	2	2
Polymicrobial (<i>n</i> =37)	<i>C. albicans</i> + <i>C. glabrata</i>	16	32
	<i>C. albicans</i> + <i>C. parapsilosis</i>	3	6
	<i>C. albicans</i> + <i>C. tropicalis</i>	2	4
	<i>C. albicans</i> + <i>C. krusei</i>	1	2
	<i>C. albicans</i> + <i>C. kefir</i>	1	2
	<i>C. tropicalis</i> + <i>C. kefir</i>	1	2
	<i>C. tropicalis</i> + <i>C. glabrata</i>	1	2
	<i>C. tropicalis</i> + <i>C. norvegensis</i>	1	2
	<i>C. glabrata</i> + <i>C. parapsilosis</i>	2	4
	<i>C. glabrata</i> + <i>C. kefir</i>	1	2
	<i>C. krusei</i> + <i>C. kefir</i>	2	4
	<i>C. krusei</i> + <i>C. parapsilosis</i>	1	2
	<i>C. kefir</i> + <i>C. parapsilosis</i>	1	2
	<i>C. kefir</i> + <i>C. norvegensis</i>	1	2
	<i>C. albicans</i> + <i>C. tropicalis</i> + <i>C. glabrata</i>	2	6
	<i>C. albicans</i> + <i>C. parapsilosis</i> + <i>C. krusei</i>	1	3
Total		502	542

can mimic the morphotype of *C. glabrata* (pale-blue colonies). The other yeast species produce colonies which have a similar colour and texture to those obtained on SDA. Conventional identification tests such as Bichrolatex *albicans*, Krusei Color and GLABRATA RTT, API 32C, Auxacolor 2 and antifungal susceptibility tests (E-test and Fungitest) can be performed directly using colonies isolated

on CS4. CS4 also allowed the growth of other yeast or yeast-like species or filamentous fungi; however, subculture on Czapek agar or SDA is necessary for the identification of these moulds.

In conclusion, this prospective study demonstrated that CS4 is an effective, selective and useful new chromogenic

Table 2. Primary cultures: direct identification of *C. albicans* isolated from monomicrobial and polymicrobial specimens using CS4 and CA plates

Incubation time (h) at 37 °C	Cumulative no. (%) of <i>C. albicans</i> isolates			No. of negative cultures	
	Conventional identification	CS4	CA	CS4	CA
24		122* (31.2)	129* (32.9)		
		9† (2.2)	9† (2.5)		
48		325* (82.9)	325* (82.9)		
		7† (1.7)	0† (0)		
72	392	361* (92.1)	357* (91.1)	20 (5.5)	35 (9.6)
		11† (2.7)	0† (0)		

*Directly identified.

†Remaining white.

Table 3. Primary cultures: presumptive identification of *C. glabrata*, *C. tropicalis* and *C. krusei* in monomicrobial/polymicrobial specimens using CS4 and CA plates after 48 h incubation at 37 °C

Yeast species	Conventional identification	Cumulative no. (%) of <i>Candida</i> species identified by:	
		CS4	CA
<i>C. glabrata</i> *	60	48 (80)	45 (75)
<i>C. tropicalis</i> †	25	17 (68)	19 (76)
<i>C. krusei</i>	12	11 (91.6)	10 (83.3)
Other yeast species	53	–	–

*Three false-positive identifications were observed with CS4 (two *C. kefyr* and one *C. parapsilosis*) and four false-positives with CA (two *C. kefyr*, one *C. guilliermondii* and one *C. parapsilosis*).

†Three false-positive identifications were observed with CS4 (one *C. guilliermondii* and two *C. kefyr*).

medium for the presumptive identification of the most frequently isolated *Candida* species.

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