

# Routine use of CHROMagar Candida medium for presumptive identification of *Candida* yeast species and detection of mixed fungal populations

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**Objective:** To assess the value of the new differential culture medium CHROMagar Candida for routine investigation of clinical specimens.

**Methods:** During a whole year, 6150 clinical samples were plated on CHROMagar Candida medium. After incubation, the green colonies were considered to be *Candida albicans*. The colonies of other colors were identified using Bichrolatex-krusei, or by their assimilation pattern on ID 32C test strips and their morphology on rice cream-agar-Tween.

**Results:** Among the 6150 clinical samples, 1643 were positive for fungi. *Aspergillus fumigatus* and *Geotrichum* sp. were the predominant filamentous fungi isolated. *Candida albicans* was the most common species isolated (1274 of the positive samples; 77.5%), and *Candida glabrata* was the second most common yeast isolated (174 positive samples; 10.6%). Other yeast species were detected at lower frequencies, mainly *Candida tropicalis* (3.8%), *Candida krusei* (2.7%), *Saccharomyces cerevisiae* (2.7%) and *Candida kefyr* (2.3%), and 16 samples revealed a lipophilic species, *Malassezia furfur*. Mixed fungal populations accounted for 14.7% of the positive samples. Two or more yeast species were detected in 206 of the 242 specimens containing mixed fungal populations, and five yeast species were detected in one sample. Additionally, we did not observe significant differences in the isolation of yeasts or filamentous fungi from the 366 samples simultaneously plated on CHROMagar Candida and Sabouraud dextrose agar. Close agreement between the two culture media was observed for 89.9% of these samples.

**Conclusions:** CHROMagar Candida medium was shown to be extremely helpful in a routine clinical mycology service, facilitating the detection of mixed cultures of yeasts and allowing direct identification of *C. albicans*, as well as rapid presumptive identification of the other yeasts: *C. glabrata*, *C. tropicalis*, *C. krusei* and *S. cerevisiae*. This chromogenic medium thus appears to be suitable as a primary culture medium, particularly for the mycologic surveillance of immunocompromised patients.

**Key words:** Yeasts, differential culture media, CHROMagar Candida medium

During the past two decades, the incidence of candidiasis has increased markedly with the advent of new diseases such as the acquired immunodeficiency syndrome and the development of immunosuppressive therapy [1,2]. The increase in the length of hospitalization and in the severity of illness of hospitalized patients,

added to the widespread use of broad-spectrum antibiotics and of invasive devices or procedures (e.g. parenteral hyperalimentation and placement of indwelling catheters), has also resulted in an increase in the rate of candidosis. Among the causative agents of these mycoses, *Candida albicans* is the most common species isolated, representing between 60% and 80% of all clinical isolates. However, recent data indicate a marked shift in the distribution of yeast species [2]. More than 30% of nosocomial infections are due to non-*C. albicans* species, such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, which presently comprise with *C. albicans* nearly 95% of the clinical isolates of yeasts. For example, in the USA, the prevalence of *C. albicans* in

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septicemia declined from 87% of the total yeast isolates in 1987 to a low of 31% in 1992 with concomitant increases of *C. glabrata* (from 2% to 26%), *C. tropicalis* (2% to 24%) and *C. parapsilosis* (9% to 20%) [3]. Furthermore, other yeast species have emerged as pathogens in immunocompromised patients [2,4–6]: *C. krusei*, *C. lusitaniae*, *C. lipolytica* and species belonging to the genus *Trichosporon* (*T. cutaneum*) or *Hansenula* (*H. anomala*).

Isolation and prompt identification of the infecting microorganism are required for early antifungal therapy. Rapid presumptive identification of *C. albicans* has been based on the demonstration of germ tubes following incubation at 37°C for 3 h in pooled human or animal serum [7]. However, this test, which is time-consuming, may lead to both false-positive and false-negative results [8–13]. Full yeast identification by other current methods, such as the production of chlamydospores on rice cream-agar-Tween, and the ability to utilize sugars or to grow in the presence of cycloheximide, takes 48 to 72 h.

Moreover, in addition to a dominant species, clinical samples may contain other associated yeast species which may come to dominate under selective pressure by an antifungal agent. Sabouraud dextrose agar (SDA) medium is widely used in medical mycology for the isolation and routine subculture of most common fungal pathogens [14]. However, precise identification of yeasts by colonial appearance on this culture medium is not possible in practice, and it is difficult to detect mixed cultures. Several primary culture media containing fluorogenic or chromogenic substrates specific for *C. albicans* have recently been developed. Among them, CHROMagar Candida is a new differential culture medium intended for the isolation and rapid presumptive identification of the most common clinically important yeast species. The basic performance of this culture medium has been extensively studied by Odds and Bernaerts [15] in an evaluation including 726 yeast isolates, of which most were collection strains. Its accuracy in clearly indicating colonies of *C. albicans* has recently been confirmed by different groups [16–19] using limited numbers of reference strains or of clinical isolates. This study was intended to assess the value of this culture medium in a very practical way in routine investigation of clinical specimens.

## MATERIALS AND METHODS

### Culture media

CHROMagar Candida was supplied from CHROMagar (Paris, France) as a dehydrated powder in preweighed batches for the preparation of 1000-mL volumes. The

medium (which comprises, in grams per liter: peptone, 10; glucose, 20; chromogenic mix, 2; chloramphenicol, 0.5; agar, 15) was prepared according to the manufacturer's instructions and dispensed into 95-mm-diameter Petri dishes (20 mL each). Plates were stored at 4°C and used within 2 weeks. SDA (containing in g per liter: peptone, 10; yeast extract, 5; glucose, 20; chloramphenicol, 1; agar, 20) was prepared in our laboratory.

### Patients and clinical specimens

This study involved 6150 clinical samples collected during a whole year and included the regular follow-up of patients and case studies of potential nosocomial infections by *C. lusitaniae* in a pediatric hospital department. The patients are classified into three groups: (1) HIV-positive patients, generally examined by mouth swabs (corresponding to 384 samples); (2) non-HIV immunocompromised patients from two hematology departments in Angers Hospital who were surveyed systematically each week for fungal risk by means of nostril, mouth and ear swabs, and by microbiological analysis of feces and urine, or patients hospitalized for pulmonary diseases and subjected to sputum or bronchoalveolar fluid analysis (3291 samples); and (3) non-immunocompromised patients displaying symptoms of bacterial or mycologic infection, or yeast allergy, and usually examined by vaginal swabs and further urine or feces analysis (2475 samples).

### Study design

Nostril, mouth, ear or vaginal swabs, as well as 100- $\mu$ L aliquots for the urine samples, were plated on CHROMagar Candida medium (CHROMagar, Paris, France). Feces were suspended in 10 volumes of sterile distilled water, and 100- $\mu$ L aliquots were streaked onto CHROMagar Candida. Likewise, 10- $\mu$ L aliquots of sputum samples were directly applied on CHROMagar Candida plates, and simultaneously inoculated on SDA after pretreatment with an equal volume of a mucolytic agent (Digest-EUR; Eurobio, Les Ulis, France). Centrifugation pellets of bronchoalveolar fluids and homogenized biopsies were cultured simultaneously on SDA and CHROMagar Candida plates. After inoculation, the plates were incubated at 37°C and examined daily for 7 days.

Filamentous fungi were identified on the basis of their macroscopic and microscopic morphology. Among yeast isolates, the green colonies on CHROMagar Candida were considered to be *C. albicans* on the basis of the manufacturer's instructions and previous reports [15–19]. Colonies on SDA were identified as *C. albicans* using Bichrolatex albicans (Laboratoires Fumouze,

**Table 1** Mixed populations of fungi detected on CHROMagar Candida medium in the three patient groups

Study patient group	Positive cultures (total number of samples)	Number of samples containing several (n) associated fungi			
		n = 2	n = 3	n = 4	n = 5
HIV patients	219 (384)	30	2		
Non-HIV immunocompromised patients	450 (3291)	48	4		
Non-immunocompromised patients	974 (2475)	142	13	2	1
Total	1643 (6150)	220	19	2	1

Asnières, France). Colonies of other colors from CHROMagar Candida and non-*C. albicans* yeast isolates from SDA were identified using Bichrolatex-krusei [20] or by their auxanographic characters on ID 32C test strips (bioMérieux, Marcy-l'Etoile, France) and their morphology on rice cream-agar-Tween.

## RESULTS

During the experimental period, 6150 clinical samples were examined and, of these, 1643 (26.7%) contained fungi. A total of 118 filamentous fungi and of 1793 yeast isolates was recovered. Mixed populations of fungi accounted for 14.7% of the positive samples (Table 1). Two different species associated were recovered from 220 samples: two filamentous fungi in one sample, a yeast and a filamentous fungus in 35 samples, and two yeast species in 184 samples. Among the samples containing three different species, yeasts were associated with a filamentous fungus in four cases, and three yeast species were recovered in 15 specimens. Three yeast species associated with a filamentous fungus were

recovered from two samples, and one sample contained five associated yeast species.

Our study permitted a detailed description of the different parts of the body from which fungi could be isolated and allowed us to refine this analysis species by species. The isolated filamentous fungi are presented in Table 2, and the yeasts in Table 3. The dominant species were *C. albicans* and *C. glabrata*, which accounted for 77.5% and 10.6% of the positive specimens, respectively. Other yeast species were detected at lower frequencies, mainly *C. tropicalis* (3.8%), *C. krusei* (2.7%), *Saccharomyces cerevisiae* (2.7%) and *C. kefyr* (2.3%). Surprisingly, 16 samples plated on CHROMagar Candida, a medium which does not contain lipids, revealed very small colonies of yeasts that we identified as *Malassezia furfur*, a lipophilic species. These isolates, which could not be subcultured and did not grow on ID 32C test strips, were identified on the basis of the colony morphology on primary plates, in addition to microscopic examination and colony development after addition of olive oil to the surface of the culture medium.

As shown in Table 4, mixed species of fungi were recovered from various specimens, with a relatively high frequency in pulmonary specimens (26.7% of the positive samples), and, in contrast very low frequency in genital specimens (3.8%), in which a single species, either *C. albicans* (86.3%) or *C. glabrata* (7.7%), was commonly isolated. The most common associations are summarized in Table 4. *C. albicans* was detected in 77.8% of the samples containing two yeast species. In addition, at least three different yeast species were simultaneously recovered from 18 specimens, and *C. albicans* and *C. glabrata* were recovered in association with another species in four of these samples. Of all specimens containing mixed fungal populations, *C. albicans* was detected in 79.3%, and *C. glabrata* in 35.5%, and at least two non-*C. albicans* yeast species were recovered from 9.5%. Filamentous fungi were recovered in association with yeasts from 41 out of 1643

**Table 2** Filamentous fungi detected using CHROMagar Candida medium

Fungal species	Specimens									Total number of positive samples
	Nostril (n = 709)	Ear (n = 384)	Buccal (n = 1929)	Pulmonary (n = 344)	Digestive (n = 1009)	Genital (n = 834)	Urine (n = 702)	Cutaneous (n = 127)	Other (n = 112)	
<i>Absidia</i> sp.				1						1
<i>Aspergillus fumigatus</i>	7	1	4	40			2		1	55
<i>Aspergillus flavus</i>	1			4						5
<i>Aspergillus terreus</i>				1						1
<i>Fusarium</i> sp.							1			1
<i>Geotrichum</i> sp.			5	1	47			1		54
<i>Trichophyton mentagrophytes</i>								1		1

**Table 3** Yeast species detected using CHROMagar Candida medium

Fungal species	Specimens									Total number of positive samples
	Nostril (n = 709)	Ear (n = 384)	Buccal (n = 1929)	Pulmonary (n = 344)	Digestive (n = 1009)	Genital (n = 834)	Urine (n = 702)	Cutaneous (n = 127)	Other (n = 112)	
<i>Candida albicans</i>	3	1	607	118	235	211	37	47	15	1274
<i>Candida glabrata</i>	2		57	14	43	23	27	5	3	174
<i>Candida guilliermondii</i>			5	3	4		2			14
<i>Candida holmii</i>					1					1
<i>Candida humicola</i>					1					1
<i>Candida inconspicua</i>			12	1	6				1	20
<i>Candida kefyr</i>			12	1	22	2	1			38
<i>Candida krusei</i>			25	4	12		1		3	45
<i>Candida lambica</i>					2					2
<i>Candida lipolytica</i>			1							1
<i>Candida lusitanae</i>			11	1	15	1	6		2	36
<i>Candida norvegensis</i>			2		1					3
<i>Candida parapsilosis</i>	2	1	7	5	7	1			1	24
<i>Candida pelliculosa</i>			4		1					5
<i>Candida rugosa</i>			7		4					11
<i>Candida sphaerica</i>					2					2
<i>Candida tropicalis</i>			28	15	14	2		3		62
<i>Cryptococcus neoformans</i>									2	2
<i>Malassezia furfur</i>	7	8					1			16
<i>Malassezia pachydermatis</i>						1				1
<i>Pichia</i> sp.			1		1					2
<i>Rhodotorula rubra</i>					1					1
<i>Saccharomyces cerevisiae</i>			11	3	25	2	2	1		44
<i>Trichosporon capitatum</i>			2		1					3
<i>Trichosporon cutaneum</i>			1		2					3
<i>Zygosaccharomyces</i> sp.			4		4					8

positive samples. However, the majority of the mixed populations contained only yeasts, and, for instance, the sample with five different species contained five *Candida* species which could be differentiated on CHROMagar Candida (*C. albicans*, *C. tropicalis*, *C. kefyr*, *C. inconspicua* and *C. lusitanae*).

Additionally, there were no significant differences in the isolation of yeasts or filamentous fungi for the 366 clinical samples simultaneously plated on CHROMagar Candida and SDA. Overall agreement between the two culture media was 89.9%: 189 samples were negative on both media, and among the samples

**Table 4** Main associations of fungi detected using CHROMagar Candida medium

	Specimens									Total number of positive samples
	Nostril	Ear	Buccal	Pulmonary	Digestive	Genital	Urine	Cutaneous	Other	
Positive samples	20	10	704	161	359	234	76	50	29	1643
Samples containing mixed fungal populations	1	1	93	43	79	9	4	9	3	242
Main associations detected <sup>a</sup>										
<i>C. albicans</i> + <i>C. glabrata</i>			29	6	24	5	4	5		73
<i>C. albicans</i> + <i>S. cerevisiae</i>			7	1	8	2				18
<i>C. albicans</i> + <i>C. krusei</i>			11	3	2					16
<i>C. albicans</i> + <i>A. fumigatus</i>				16						16
<i>C. albicans</i> + <i>C. tropicalis</i>			7	1	3	1		3		15
<i>C. albicans</i> + <i>Geotrichum</i> sp.			2		9			1		12
<i>C. albicans</i> + <i>C. kefyr</i>			1		5	1				7
<i>C. albicans</i> + <i>C. inconspicua</i>			4	1	1					6

<sup>a</sup>Detected in more than five samples.

**Table 5** Differences in the species detected between CHROMagar Candida and SDA plates

CHROMagar Candida	SDA	Number of positive cultures
None	<i>Alternaria</i> sp.	1 <sup>a</sup>
None	<i>Mucor</i> sp.	1 <sup>a</sup>
None	<i>Penicillium</i> sp.	1 <sup>a</sup>
None	<i>Rhizopus</i> sp.	1 <sup>a</sup>
None	<i>C. albicans</i>	6 <sup>b</sup>
None	<i>C. glabrata</i>	1 <sup>a</sup>
<i>A. fumigatus</i>	None	3 <sup>c</sup>
<i>A. flavus</i>	None	1
<i>C. albicans</i>	None	5
<i>C. glabrata</i>	None	1
<i>C. kefyr</i>	None	1
<i>A. fumigatus</i>	<i>A. fumigatus</i> + <i>C. albicans</i>	1
<i>A. fumigatus</i> + <i>C. albicans</i>	<i>A. fumigatus</i>	2
<i>A. fumigatus</i> + <i>C. albicans</i>	<i>C. albicans</i>	3
<i>C. albicans</i>	<i>A. fumigatus</i>	2
<i>C. albicans</i>	<i>A. fumigatus</i> + <i>C. albicans</i>	3
<i>C. albicans</i>	<i>Cladosporium</i> sp. + <i>C. albicans</i>	1
<i>A. fumigatus</i> + <i>C. tropicalis</i>	<i>C. tropicalis</i>	1
<i>A. flavus</i> + <i>C. albicans</i>	<i>C. albicans</i>	1
<i>A. flavus</i> + <i>C. tropicalis</i>	<i>C. tropicalis</i>	1

<sup>a</sup> Only one colony detected.

<sup>b</sup> Only one to five colonies for four of the samples.

<sup>c</sup> Only one colony for one of the samples.

positive on CHROMagar Candida and/or SDA, 140 gave identical results on the two media. Table 5 shows the differences in the species detected between CHROMagar and SDA plates.

## DISCUSSION

The presence of more than one yeast species in clinical samples is not uncommon. Yamane and Saitoh [21] as well as Samaranayake et al. [22] have reported the association of yeast species with a relatively high frequency, ranging from 8% to 15.3% of the positive cultures, varying with the nature of the samples. The prompt detection of associated yeast species and presumptive identification of the isolated yeasts may be an aid for rapid appropriate treatment decisions, in the light of differences in susceptibility of the yeast species to antifungal agents [23]. Some *Candida* species such as *C. lusitanae* are often resistant to amphotericin B; numerous isolates of *C. tropicalis* are resistant to 5-fluorocytosine; *C. glabrata* is less sensitive than other species to ketoconazole and fluconazole; and *C. krusei* exhibits innate resistance to fluconazole. Thus, there has been increasing concern during the past few years

that the extended use of ketoconazole or fluconazole as prophylactic or curative agents for mycoses may be associated with an increased incidence of *C. glabrata* and *C. krusei* infections.

According to Yamane and Saitoh [21], only 26% of mixed populations of yeasts are detected on SDA. Differential culture media have therefore been developed, based on a varying pigmentation of the colonies by reduction of an inorganic substrate added to the medium, such as phosphomolybdate [24,25], 2,3,5-triphenyltetrazolium chloride in Pagano-Levin's culture medium [26] or bismuth polysulphite in Nickerson's medium [27]. Likewise, the YM + aniline blue dye medium enables the direct identification of *C. albicans* which, on this medium, specifically forms a fluorescent metabolite that can be visualized with long-wave UV light [28]. However, the phosphomolybdate agar medium is not commercially available, and the limited color variations of the colonies on the other media (from brown to black on bismuth-based media, and from white to pink on Pagano-Levin's medium, depending on the species) may lead to an incorrect evaluation of the real number of associated species [29–32]. A reduction in the number of colonies and in their size has often been reported on Pagano-Levin's medium compared with the results on SDA. Moreover the growth of some yeast species such as *C. lusitanae*, *C. lambica* and *Cryptococcus albidus* appears to be partly inhibited on this culture medium.

More recently, differential culture media based on the detection of the *N*-acetyl- $\beta$ -D-galactosaminidase specific to *C. albicans* have been proposed: Fluoroplate Candida agar from Merck, CANDICHRM albicans from International Mycoplasma, Albicans ID from bioMérieux and CandiSelect from Diagnostics Pasteur [19,33–35]. With fluoroplate Candida agar, which requires the use of a UV lamp for interpretation, a diffusion of the fluorescent product to neighboring colonies is often observed after 48 h of incubation, which may lead to misidentification of non-*C. albicans*-associated yeasts [35]. Additionally, all these culture media differentiate only the colonies of *C. albicans* from the non-fluorescent or colorless colonies of non-*C. albicans* yeast species. With CHROMagar Candida medium, there was no delay in yeast detection and no change in the size of colonies compared with that obtained on SDA when the same clinical samples were plated onto the two culture media. Likewise, we did not observe significant differences between CHROMagar Candida and SDA with regard to the growth of filamentous fungi, thus confirming the observations of Odds and Bernaerts [15] using reference strains of dermatophytes and moulds, including some aspergilli.

Like the other chromogenic media, CHROMagar Candida allows the direct identification of *C. albicans*, characterized by the green color and the smooth aspect of the colonies. Moreover, this medium allows the presumptive identification of additional species such as *C. tropicalis* (metallic blue colonies), *S. cerevisiae* (purple colonies with a dark halo in agar) and *C. glabrata* (white or purple, smooth, glittering colonies). *C. krusei* is also easily recognizable by its pale rose, flat and spreading colonies with a broad white fringe, and its identification is rapidly confirmed by using Bichrolatex-krusei [20]. In our hands, CHROMagar Candida correctly identified 100% of *C. krusei* isolates, and the presumptively identified *C. tropicalis* isolates were always confirmed by conventional methods. However, on the basis of colony morphology and pigmentation on CHROMagar, 8.5% of *C. tropicalis* isolates were misidentified as *S. cerevisiae*. In addition, this culture medium also permitted the detection of *M. furfur*, a yeast known for its requirement for lipids. Isolation of this yeast, which usually grows on the sebaceous parts of the skin, may be surprising. However, its presence in ear and nostril specimens, but also in urine samples of patients undergoing parenteral hyperalimentation, is not uncommon. Our isolates were obtained from four young patients hospitalized in a pediatric hematology department of Angers hospital for cancer or hematologic malignancies. One of them presented a positive urine sample. Another isolate was obtained from an ear specimen in a second patient, associated with *C. parapsilosis*. A third patient presented five positive nostril swabs, and the nine other isolates were from a single patient.

With a frequency of 14.7% of the positive samples, our study confirms the previous observations of the frequency of mixed fungal populations [17,21,22]. For example, in the study of Pfaller et al. [17], 18% of the positive samples contained mixtures of yeast species, and 20% of these mixed cultures were not detected on SDA. All our samples were not inoculated in parallel on SDA. It seems, however, that the use of CHROMagar Candida greatly increases the percentage of mixed fungal populations detected in clinical samples, since, until the introduction of this chromogenic medium in our hospital laboratory, their frequency was obviously much lower (5.4% in 1993).

As has previously been observed with the use of Pagano-Levin's culture medium [21,22] or CHROMagar Candida [17], the most common association was *C. albicans* + *C. glabrata*, which was detected in 30% of the samples containing mixed fungal populations (46.5% according to Pfaller et al. [17]). However, quite a number of samples contained at least two non-*C. albicans*-associated yeasts. For example,

during the course of our routine systematic work, the following investigation was carried out. After several isolates of *C. lusitaniae* had been obtained from children in a pediatric hematology department, 60 members of the personnel of this hospital unit were investigated by mouth swab, urine and feces analysis for asymptomatic carriage of this rather rare species. In only one case was *C. lusitaniae* isolated in a mixed population including four other species: *C. albicans*, *C. tropicalis*, *C. kefir* and *C. inconspicua*. Obviously, such detailed study with naturally occurring mixed populations would have been very difficult, if at all possible, without CHROMagar Candida, which is a powerful tool for the differentiation of yeast species.

In conclusion, in spite of its greater cost (in comparison with SDA), CHROMagar Candida medium was found to be extremely helpful, allowing direct identification of *C. albicans* colonies as well as the presumptive identification of the other most common yeasts, and the easy recognition of associations of multiple yeast species. Thus this easy-to-use and time-saving medium appears to be well suited for routine use in clinical mycology laboratories.

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