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Direct Identification and Recognition of Yeast Species from Clinical Material by Using Albicans ID and CHROMagar Candida Plates

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Two chromogenic media, Albicans ID and CHROMagar Candida agar plates, were compared with a reference medium, Sabouraud-chloramphenicol agar, and standard methods for the identification of yeast species. This study involved 951 clinical specimens. The detection rates for the two chromogenic media for polymicrobial specimens were 20% higher than that for the Sabouraud-chloramphenicol agar plates. The rates of identification of *Candida albicans* for Albicans ID and CHROMagar Candida agar plates were, respectively, 37.0 and 6.0% after 24 h of incubation and 93.6 and 92.2% after 72 h of incubation, with specificities of 99.8 and 100%. Furthermore, CHROMagar Candida plates identified 13 of 14 *Candida tropicalis* and 9 of 12 *Candida krusei* strains after 48 h of incubation.

The incidence of fungal infections, particularly multiple-yeast infections, is increasing because of a rising number of immunocompromised patients and the widespread use of broad-spectrum antibiotics (2, 5, 19). Although *Candida albicans* remains the most frequently isolated yeast pathogen, other *Candida* species such as *C.* (*Torulopsis*) *glabrata*, *C. tropicalis*, and *C. krusei* are emerging as opportunistic pathogens, and these species are more resistant than *C. albicans* to antifungal agents (1, 4, 11, 14, 19). Consequently, mycoses are a growing medical problem requiring prompt diagnosis and early adapted antifungal therapy.

Two criteria for new primary culture yeast media are the capacities (i) to facilitate the differentiation of species in mixed cultures and (ii) to allow direct and rapid identification of yeasts, especially those which are resistant to antifungal agents. It is therefore understandable that chromogenic media, which appear to fulfill these requirements, are of great interest to microbiologists.

The aim of this study was to evaluate the performance of two of these media, Albicans ID (bioMérieux, Marcy l'Etoile, France) and CHROMagar Candida (CHROMagar, Paris, France), in comparison with our standard method.

A total of 951 clinical samples (394 genital, 274 stool, 74 urine, 57 ear, nose, and throat, 45 bronchoalveolar fluid, and 21 sputum specimens and 86 miscellaneous samples such as wound, eye, and skin specimens) were collected from patients at hospital clinics (Hôpital de l'Antiquaille and Hôpital de la Croix-Rousse, Lyon, France). Each nonfluid specimen was suspended in 0.85% physiological saline, and then 0.01 ml of this suspension was streaked onto Albicans ID, CHROMagar Candida, and Sabouraud-chloramphenicol agar plates. Equivalent amounts of each fluid specimen were directly applied to the media.

Albicans ID (bioMérieux) is a commercially available, ready-to-use medium which was previously described (15) (bio-Thione, 2 g; yeast extract, 6 g; sodium hydrogenophosphate, 0.5 g; chromogenic substrate [hexosamine], 0.05 g; *N*-2-acetamidoimino-diacetic acid monosodium salt buffer, 0.5 g; gentamicin sulfate, 0.1 g; chloramphenicol, 0.05 g; and agar, 14 g; made up to 1 liter with distilled water [pH 6.6]) and which, after hydrolysis by the corresponding enzyme, allows the specific identification of *C. albicans* colonies on the basis of their blue color and smooth appearance. CHROMagar Candida (CHROMagar) is a new differential isolation medium (peptone, 10 g; glucose, 20 g; agar, 15 g; chloramphenicol, 0.5 g; and chromogenic mixture, 2 g; made up to 1 liter with distilled water) which, after hydrolysis by the corresponding enzyme, allows the specific identification of *C. albicans* colonies on the basis of their green color, *C. tropicalis* colonies on the basis of their blue color surrounded by a pink halo, and *C. krusei* colonies on the basis of their pink color and downy appearance. It was supplied as a white powder in preweighed batches for the preparation of 1,000-ml volumes and was prepared in our laboratory according to the manufacturer's instructions. The plates were stored in the dark at 4°C for up to 1 month. Sabouraud-chloramphenicol agar (Diagnostics Pasteur, Marnes la Coquette, France), a Sabouraud glucose agar containing chloramphenicol (0.05 g/liter) that is used in our clinical laboratory for the growth and isolation of yeasts, was inoculated in parallel with the other two media.

For all three agars, the morphologically different colonies were picked and identified by conventional methods: germ tube induction in foal serum (Diagnostics Pasteur) at 37°C for 2 to 4 h; chlamydospore formation on rice-agar-Tween medium (bioMérieux); and, if necessary, assimilation pattern with the Auxacolor yeast identification panel (Diagnostics Pasteur) (16).

Reading of the plates and interpretation of the results were conducted after 24, 48, and 72 h of incubation at 30°C. To control for observer bias in the reading of the results, determination of the color and enumeration of the different colonies grown on Albicans ID, CHROMagar Candida, and Sabouraud-chloramphenicol agar plates were performed by three different readers.

Direct identification of *C. albicans* on the two chromogenic media was analyzed in terms of sensitivity [number of true positives/(number of true positives + number of false negatives)] and specificity [number of true negatives/(number of true negatives + number of false positives)].

Of the 951 specimens, 498 did not give cultures and 453 gave

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TABLE 1. Yeast and yeast-like strains detected from 951 clinical specimens after 72 h of incubation at 30°C on Sabouraud-chloramphenicol, Albicans ID, and CHROMagar Candida plates

Species	No. of yeast strains detected			
	Sabouraud-chloramphenicol	Albicans ID	CHROMagar	Any media[a]
Candida albicans	229	266	261	281
Geotrichum candidum	116	118	116	135
Candida (*Torulopsis*) *glabrata*	73	84	90	94
Candida kefyr	13	11	20	23
Candida tropicalis	7	12	14	17
Candida krusei	11	12	12	14
Saccharomyces cerevisiae	5	6	9	9
Candida parapsilosis	6	6	6	7
Candida inconspicua	1	4	4	6
Candida lusitaniae	3	4	5	5
Candida lipolytica	1	5	3	5
Candida famata	2	4	4	4
Candida sphaerica	1	2	3	3
Rhodotorula spp.	1	0	2	3
Candida zeylanoides	2	1	1	2
Saccharomyces unisporus	0	1	1	1
Candida colliculosa	1	0	1	1
Candida robusta	1	1	1	1
Candida pentolopesii	0	1	0	1
Candida catenulata	0	0	1	1
Cryptococcus laurentii	0	1	1	1
Saccharomyces spp.	1	0	1	1
Total	474	539	556	615

[a] Total number of yeast and yeast-like strains detected on at least one of the three media.

cultures that yielded one or several yeast strains (329 monomicrobial cultures and 124 polymicrobial cultures). A total of 615 yeast strains encompassing 22 species were isolated on at least one of the three media (Table 1).

The detection rates for the three media after 72 h of incubation at 30°C for the 329 monomicrobial specimens and the 124 specimens containing two or more species are detailed in Table 2. For the monomicrobial specimens, for all instances in which there was no growth on one medium, fewer than six yeast colonies were observed on the other media. Yeast strains which were not detected on one or two of the media belonged to 11 different species (Table 3) and were isolated from various samples. There was no apparent relationship between the sample type, strain identification, and the absence of growth on any of the media.

The identification of *C. albicans* by the two chromogenic media after 24, 48, and 72 h of incubation is detailed in Table 4. After 72 h of incubation at 30°C, 263 of the 281 *C. albicans* strains growing on at least one of the three media were identified by their blue pigmentation on Albicans ID, and 259 of 281 were identified by their green pigmentation on CHROMagar Candida plates (sensitivities, 93.6 and 92.2%, respectively). One false-negative *C. albicans* strain was common to both chromogenic media. Only one false-positive strain (*C. tropicalis* colonies with pale blue centers and white edges) was found on Albicans ID agar plates, and none were found on CHROMagar Candida plates (specificities, 99.8 and 100%, respectively). Of the 14 *C. tropicalis* strains detected on CHROMagar Candida plates, 13 were correctly identified directly on the primary plates on the basis of blue colonies surrounded by a pink halo. After 48 h of incubation, one *Candida catenulata* strain gave colony color similar to that of *C. tropicalis*. Among the 12 *C. krusei* strains detected, 9 were correctly identified by their pink, downy colonies. No false-positive strain was detected. These typical appearances were described previously (6, 12). The false-negative *C. albicans*, *C. tropicalis*, and *C. krusei* strains were all isolated from stool samples. The false-negative *C. tropicalis* and two false-negative *C. krusei* strains were subcultured on a second CHROMagar Candida plate. The strains then showed their typical appearance.

Thirty bacterial strains (24 *Escherichia coli*, 1 *Klebsiella pneumoniae*, 1 *Enterobacter aerogenes*, and 4 *Pseudomonas aeruginosa* strains) mainly from stool samples grew on CHROMagar Candida plates, while four bacterial strains (2 *E. coli*, 1 *Enterococcus faecalis*, and 1 *P. aeruginosa* strain) grew on Albicans ID agar plates. All these bacterial colonies could be easily distinguished from yeast colonies by their glossy appearance.

Previous studies have shown the usefulness of chromogenic media for the detection and direct identification of *C. albicans* (3, 8, 10, 15, 17, 18). Only a few authors (6, 9, 12) have studied CHROMagar Candida, a recently developed medium which allows the presumptive identification of three *Candida* species. These studies focused mainly on cultures from strain collections with only a limited number of clinical specimens. Our study, based on 951 clinical specimens, confirms that the results obtained with the chromogenic media were superior to those obtained with the Sabouraud-chloramphenicol reference medium in terms of the detection of yeasts, especially in the case of mixed-yeast cultures. This is due to the ability of the chromogenic media to differentiate between *Candida* species directly at the isolation stage, simply by the different colors of the colonies. The detection rates obtained with Albicans ID and CHROMagar Candida plates for the 96 specimens containing two species were similar (86.5 and 84.9%, respectively) since 89% of these mixed cultures contained either the colored colonies typical of *C. albicans* (73%) or the downy colonies typical of *Geotrichum candidum* (16%). CHROMagar Candida

TABLE 2. Detection of yeast and yeast-like strains in monomicrobial and polymicrobial specimens after 72 h of incubation at 30°C

Type of specimens	No. of specimens	No. (%) of yeast strains detected			
		Any media[a]	Sabouraud-chloramphenicol	Albicans ID	CHROMagar Candida
Polymicrobial	124	286	178 (62.2)	238 (83.2)	243 (85.0)
2 species	96	192	133 (69.3)	166 (86.5)	163 (84.9)
>2 species	28	94	45 (47.9)	72 (76.6)	80 (85.1)
Monomicrobial	329	329	296 (90.0)	301 (91.5)	313 (95.1)
Total	453	615	474 (77.1)	539 (87.6)	556 (90.4)

[a] Total number of yeast and yeast-like strains detected on at least one of the three media.

TABLE 3. Yeast and yeast-like strains from the 329 monomicrobial specimens not detected on one or two media after 72 h of incubation at 30°C

Species	No. of yeast strains not detected		
	Sabouraud-chloramphenicol	Albicans ID	CHROMagar Candida
Candida albicans	15	9	5
Geotrichum candidum	5	8	7
Candida (*Torulopsis*) *glabrata*	4	4	2
Candida famata	2	0	2
Saccharomyces cerevisiae	2	1	0
Candida tropicalis	1	2	0
Candida krusei	0	1	1
Candida inconspicua	1	1	0
Candida kefyr	1	0	0
Rhodotorula spp.	1	2	1
Cryptococcus laurentii	1	0	0
Total	33	28	16

plates showed the best sensitivity of detection for both monomicrobial and polymicrobial cultures, followed closely by Albicans ID agar plates. The superiority of CHROMagar Candida plates in revealing mixtures of yeast species was shown by Odds and Bernaerts in an earlier study (12). Paugam et al. (13) have shown the clinical significance of using this medium, which allows a broader view of mixed cultures from specimens from immunocompromised patients.

In this comprehensive comparative study as well as in a previous one (7), our results show that even if the colonies were smaller on Albicans ID agar than on Sabouraud-chloramphenicol agar, their growth performance, as seen with monomicrobial specimens, was slightly better than that obtained with Sabouraud-chloramphenicol agar. Concerning the identification of *C. albicans*, after 24 h of incubation Albicans ID agar plates showed the best performance, but after 48 h of incubation CHROMagar Candida and Albicans ID agar plates showed similar abilities. The good results obtained with Albicans ID and CHROMagar Candida plates in terms of sensitivity and specificity confirm previous findings (3, 8, 12, 15, 18). Even if CHROMagar Candida plates did not identify *C. albicans* as rapidly as Albicans ID agar plates, the medium did provide the correct identification of *C. tropicalis*, which is the main source of false-positive identifications on most other chromogenic media, and *C. krusei*, which is increasingly involved in human diseases and in antifungal resistance (19). The fact that all the false-negative strains that were observed on the two chromogenic media were from stool samples suggests that the nature of the specimens might affect enzyme hydrolysis.

In conclusion, Albicans ID and CHROMagar Candida plates, which provided improved detection of yeasts and allowed the direct identification of 42.8 and 45.7%, respectively, of the 615 strains detected in this study, can be used as primary isolation and differentiation media for clinical specimens. Although detailed cost-benefit studies were not carried out, it seems clear that these media are economical in terms of labor and time. Moreover, their cost would be more than offset by the decreased need for secondary biochemical tests.

TABLE 4. Direct identification of *Candida albicans* using Albicans ID and CHROMagar Candida plates

Incubation time (h) at 30°C	Cumulative no. (%) of *C. albicans* isolates directly identified		
	Conventional identification	Albicans ID	CHROMagar Candida
24		104 (37.0)	17 (6.0)
48		254 (90.4)	248 (88.3)
72	281	263 (93.6)	259 (92.2)

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