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Evaluation of Candida ID, a New Chromogenic Medium for Fungal Isolation and Preliminary Identification of Some Yeast Species

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Candida ID, a new chromogenic medium, allows identification of *Candida albicans* (blue colonies) and preliminary identification into a group of four species (pink colonies). In comparison with Albicans ID2 and Sabouraud gentamicin chloramphenicol on 446 fungal strains, Candida ID allowed the isolation of more species than Albicans ID 2 (95.5% versus 91.2%).

Mycoses are a growing medical problem requiring prompt diagnosis with species identification and early adapted antifungal therapy (3, 7, 11, 14). The importance of identifying the pathogen quickly (20), as well as the difficulty in detecting mixed cultures in the same plate with the traditional Sabouraud glucose agar medium (2), has fostered the development of differential media for the identification of yeasts (1, 5, 6, 8, 12, 18, 19, 22). Candida ID (CAID) (bioMérieux, Marcy l'Etoile, France) is a recently commercialized, ready-to-use medium. CAID was developed to improve the isolation of yeasts, the identification of *Candida albicans* and *C. dubliniensis*, the detection of mixed cultures, and the preliminary identification of other species (*C. tropicalis, C. guilliermondii, C. kefyr*, and *C. lusitaniae*, which produce pink colonies). Further testing has to be performed to separate these four species. In our study, CAID was compared with Albicans ID2 (AID2) (bioMérieux) and Sabouraud gentamicin chloramphenicol agar (SGC) using 307 collection strains and 139 strains from 220 clinical samples.

A total of 307 fungal strains (247 yeasts and 60 filamentous fungi) from bioMérieux and Grenoble hospital collections were tested on the three media. The collection strains were subcultured on Sabouraud agar plates and incubated for 2 to 5 days at 35 or 27°C according to the species. For all isolates, a suspension of the organisms was made in physiological saline and seeded onto three plates (CAID, AID2, and SGC). The plates were incubated at 35°C except for *Penicillium* and *Fusarium* spp., which were incubated at 27°C. Equivalent amounts of each of the 220 clinical samples were directly applied to the three media and incubated at 35°C. The reading of the plates and interpretation of the results were carried out after 24, 48, and 72 h of incubation. The determination of the color, the number, and the diameter of the different colonies grown on CAID, AID2, and SGC was performed at each reading step. The colonies showing different morphologies on media inoculated with clinical samples were picked up and identified by conventional mycological methods (15): Bichrolatex albicans, Krusei color (Fumouze, Levallois-Perret, France) (13), ID32C (bioMérieux), and macroscopic and microscopic observations. The colony detections on CAID, AID2, and SGC were compared and analyzed in terms of sensitivity (number of true positives/number of true positives plus the number of false negatives) and specificity (number of true negatives/number of true negatives plus the number of false positives). The data were statistically analyzed by χ^2 test. *C. albicans* identification on CAID and AID2 was analyzed in terms of sensitivity and specificity using the described conventional mycological methods as a reference.

CAID supported the growth of more strains (426 of 446, 95.5%) than AID2 (407 of 446, 91.2%) and as many as SGC (426 of 446, 95.5%) (Tables 1 and 2). The differences between CAID and AID2 were statistically significant ($P < 0.025$). Among the 220 clinical specimens, 103 gave positive cultures (43 stools, 14 tracheal fluids, 14 bronchoscopic aspirations, 30 sputum specimens, and 2 miscellaneous samples such as drain fluid) (Table 2). For the 31 polyfungal specimens, AID2 and CAID supported the growth of more fungus strains (62 of 67, 92.5%) than SGC (53 of 67, 79.1%), with statistically significant differences ($P < 0.05$). The sensitivities of CAID and AID2 for detection and differentiation of *C. albicans* were 97.6% (125 of 128) and 100% (128 of 128), respectively, and the specificities were 89.7 and 82.7%, respectively. The colonies of 17 among 36 isolates of *C. tropicalis* were blue on AID2 but not on CAID. CAID and AID2 did not enable differentiation between *C. albicans* and *C. dubliniensis*. On CAID, colonies of *C. tropicalis* (32 of 36), *C. lusitaniae* (10 of 10), *C. guilliermondii* (9 of 10), and *C. kefyr* (14 of 14) were pink after 72 h of incubation and needed complete identification. The time to detection of fungi growing on all three media (388 strains) were determined. After 24 h of incubation at 35°C, 90.7% (352 of 388) of the fungus strains were detected on SGC, 86.3% (335 of 388) were detected on AID2, and 89.7% (348 of 388) were detected on CAID. The average sizes of the colonies of collection strains after 24 h of incubation on SGC, AID2, and CAID were 0.63 ± 0.46, 0.86 ± 0.56, and 0.94 ±

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TABLE 1. Colonial coloration observed after 72 h of incubation on the three media. (SGC, AID2, and CAID) inoculated with 307 collection strains

Species	No. of strains observed on[a]:							
	Any media	SGC (white)	AID2		CAID			
			White	Blue	White	Blue	Pink	Other colors
Aspergillus flavus	5	5*	5*		5*			
Aspergillus fumigatus	9	9*	9*		9*			
Aspergillus nidulans	1	1*	1*		1*			
Aspergillus niger	5	5*	5*		5*			
Aspergillus terreus	3	3*	3*		3*			
Candida albicans	50	50		50		50		
Candida colliculosa	4	4	1		2			
Candida dubliniensis	10	10		10		10		
Candida famata	5	5			2			
Candida glabrata	10	10	10		10			
Candida guilliermondii	10	10	10		1		9	
Candida inconspicua	5	5	5		5			
Candida intermedia	5	5	3		2		3	
Candida kefyr	10	10	9	1			10	
Candida krusei	10	10	10		10			
Candida lipolytica	5	5	5		5			
Candida lodderae	1	1		1		1		
Candida lusitaniae	9	9	8	1			9	
Candida norvegensis	5	5	3	2	3		1	1
Candida parapsilosis	10	10	10		10			
Candida pelliculosa	5	5	5		2		3	
Candida pulcherrima	5	5	5				5	
Candida rugosa	5	5	3	2	4	1		
Candida sake	1	1	1		1			
Candida sphaerica	5	5	3		1		4	
Candida tropicalis	29	29	14	15	1		27	1
Candida utillis	4	4	2		2		2	
Candida zeylanoides	1				1			
Cryptococcus albidus	4	4			2			
Cryptococcus humicolus	4	4	3		4			
Cryptococcus laurentii	3	3		1	1		1	
Cryptococcus neoformans	10	10	8	1	3		6	1
Cryptococcus uniguttulatus	1	1						
Fusarium sp.	2	2*	2*		2*			
Geotrichum candidum	5	5	5		5			
Geotrichum capitatum	5	5	5		5			
Penicillium sp.	4	4*	4*		4*			
Rhodotorula rubra	4	1	1		4			
Saccharomyces cerevisiae	8	8	6		6			
Scedosporium sp.	1	1*	1*		1*			
Trichosporum cutaneum	4	4		3	1	2		
Trichosporon asahii	5	5	1	4		5		
Trichosporon asahii/asteroides	4	4	1	3	1	3		
Trichosporon inkin	5	5	1	4		5		
Trichosporon mucoides	10	10	1	8	1	8		1
Zygosaccharomyces fermentati	1	1	1				1	

[a] Filamentous fungi, which initially appeared as white and moist colonies but then changed appearance depending on the species, are indicated by an asterisk. Total strains observed were as follows: any media, 307; SGC, 301; AID2, 274; CAID, 293.

0.55 mm, respectively. Bacteria were isolated from 23 of the 220 clinical samples (10.5%) on at least one of the three media. The numbers of bacterial strains isolated on SGC, AID2, and CAID were 14, 14, and 20, respectively.

CAID was developed to obtain better results than with AID2 (4, 9, 10, 17, 21). Our study showed that CAID allowed a better detection of different types of collection strains than AID2 (95.4 and 89.2%, respectively) and that the results from clinical samples were equivalent with CAID and AID2 (95.7%). The collection strains detected only on CAID belonged to species that were not isolated from the clinical samples in our study (*C. colliculosa, C. famata, C. intermedia, C. sphaerica, C. utilis, C. zeylanoides, Cryptococcus humicolus, Cryptococcus laurentii*, and *Rhodotorula rubra*). The other advantage of CAID related to the specificity of *C. albicans* identification. No *C. tropicalis* colonies were blue on CAID in contrast to what happens on AID2. *C. lodderae* and *C. rugosa*, which produce blue colonies on CAID, were rarely isolated from clinical specimens (16), blue *Trichosporon* colonies were easily differentiated from *C. albicans* by their macroscopic morphology, while *C. dublinensis* was not distinguished from *C. albicans*. Furthermore, when different yeast species were mixed in a single sample and plated out on CAID, the distinction between colony colors and forms was extremely easy to do.

TABLE 2. Colonial coloration observed after 72 h of incubation on the three media (SGC, AID2, and CAID) inoculated with 103 clinical samples

Species	No. of fungal strains detected on[a]:						
	Any media[b]	SGC (white)	AID2		CAID		
			White	Blue	White	Blue	Pink
Aspergillus fumigatus	11	6[f]	8[f]		10[f]		
Candida albicans	78	76		78	1[c]	75	
Candida glabrata	10	10	10		10		
Candida kefyr	4	4	4				4
Candida krusei	7	7	6		7		
Candida lusitaniae	1	1	1				1
Candida norvegensis	1				1		
Candida parapsilosis	3	3	3		3		
Candida tropicalis	7	5	5	2[d]	2[e]		5
Geotrichum candidum	7	6	7		6		
Paecilomyces sp.	2		1[f]		1[f]		
Saccharomyces cerevisiae	8	7	8		7		

[a] Total fungal strains detected: any media, 139; SGC, 125; AID2, 133; CAID, 133.
[b] Total number of fungal strains detected on at least one of the three media.
[c] The presence of bacteria has inhibited the coloration of yeast colonies.
[d] The colonies were blue with a white center.
[e] Case 1, the presence of bacteria has inhibited the coloration of yeast colonies, case 2, the colonies were pink after subculture.
[f] Filamentous fungi (initially appeared as white and moist colonies but then changed appearance depending on the species).

Discrimination of *C. kefyr, C. lusitaniae, C. tropicalis*, and *C. guilliermondii* from other yeasts by their pink colonies facilitates their definitive identification particularly for *C. guilliermondii* which has the same profile as *C. famata* on the ID32C strip. On CAID, the colonies of *C. guilliermondii* were pink while the colonies of *C. famata* were white; thus, no other complementary test (different from ID32C) was necessary.

In conclusion, our study shows that CAID is an effective, ready-to-use medium for the isolation of fungi and identification of *C. albicans* from samples in clinical laboratories.

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