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PERFORMANCE OF THE ALBICANS ID2® CHROMOGENIC MEDIUM FOR RAPID IDENTIFICATION OF *CANDIDA ALBICANS*

Patricio Godoy-Martínez; Ana C. Azevedo; Viviane Reis; Thelma Alves; Leila P. Almeida; Arnaldo L. Colombo*

Laboratório Especial de Micologia, Disciplina de Infectologia, Universidade Federal de São Paulo, São Paulo, SP, Brasil

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ABSTRACT

The aim of our study was to evaluate the accuracy of the chromogenic media Albicans ID2® (bioMérieux, France) for the identification of *Candida albicans* among 330 yeast strains. All *C. albicans* (100) and *C. dubliniensis* (20) strains exhibited blue color when cultured on Albicans ID2®. However, the blue color was also exhibited by cultures of *C. rugosa* (30/30) and *C. tropicalis* (3/50) isolates.

Key words: *Candida albicans*, chromogenic medium, Albicans ID2® media

During the past decade, there has been increasing recognition of emerging fungal pathogens. The cause of the emergence of different new fungal pathogens is not completely understood. The changing spectrum of invasive mycoses is probably secondary to a combination of factors including the substantial improvements in the management of malignant diseases, advances in critical care and organ transplantation, the increasing number of patients undergoing invasive medical procedures and the selective pressure of antimicrobial drug use (9,19).

Although *Candida albicans* still accounts for most of the species isolated from yeast-infected patients, other *Candida* species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* are emerging as opportunistic pathogens (13,15). Of interest, invasive infection due to non- *albicans* species may be refractory to therapy with conventional antifungal agents (1).

Traditionally, rapid identification of *C. albicans* depends on the germ tube test, which can identify *C. albicans* strains in 2h since the fungus produces germ tubes during growth at 37ºC in serum. However, up to 5% of the *C. albicans* isolates have been reported as germ tube negative, and non-*albicans* isolates may produce some structures which can be misinterpreted as germ tubes (12,17).

Otherwise, this method is time consuming and requires manipulation of human or animal serum. Alternative methods for quick *C. albicans* identification include the use of chromogenic media, as well as simple and rapid biochemical tests for detection of specific enzymes (2,11). Albicans ID2® chromogenic medium (bioMérieux, Marcy l'Etoile, France) has been developed and marketed for the identification of *C. albicans* (blue colonies). This assay is based on a chromogenic indolyl glucosaminide substrate, which is hydrolyzed by *C. albicans* to give a turquoise or blue color (4,12). The purpose of this study was to evaluate the accuracy of Albicans ID2® plates to identify *C. albicans* strains among yeasts different species with clinical relevance.

A total of 330 yeast isolates obtained from the fungal culture collection of the Laboratório Especial de Micologia, UNIFESP-EPM, was used to assess the accuracy of Albicans ID2® in the identification of *C. albicans* strains. All isolates were obtained from clinical material and included the following strains: *C. albicans* (100), *C. dubliniensis* (20), *C. tropicalis* (50), *C. glabrata* (30), *C. rugosa* (30), *C. parapsilosis* (20), *C. krusei* (20), *C. lusitaniae* (20), *C. guilliermondii* (20), *Cryptococcus neoformans* (10), *Trichosporon* spp. (10). The isolates were identified by standard methods, except for *C. dubliniensis* strains that were identified by molecular methods RAPD (random amplified polymorphic DNA analysis).

The purity and viability of original yeast cultures were checked by plating on CHROMagar Candida (CHROMagar Microbiology Paris, France). *C. albicans* and *C. dubliniensis* isolates were screened by their ability to produce green colonies on CHROMagar Candida and chlamydoconidia on corn meal-Tween 80 agar (Difco laboratories, Detroit, USA). All positive chlamydoconidia isolates

*Corresponding Author. Mailing address: Laboratório Especial de Micologia, Disciplina de Infectologia, Universidade Federal de São Paulo, Rua Napoleão de Barros, 740 - 7º andar. 04023-062, São Paulo, SP, Brasil. Fax: (+5511) 5083-0806, E-mail: colomboal@terra.com.br

were submitted to growth test at 42ºC on Sabouraud dextrose agar. The isolates that did not grow at 42ºC were genotyped by randomly amplified polymorphic DNA (RAPD) analysis using the oligonucleotide primers CDU (5'GCG ATC CCC A3') and B14 (5'GAT CAA GTC C3') in order to confirm the identification of *C. dubliniensis* (18,14). *Candida* non-*albicans* isolates were identified on the basis of their micromorphology on corn meal-Tween 80 agar and biochemical tests using the commercial system ID 32C, bioMérieux Marcy l'Etoile, France (3).

Suspensions of all isolates were prepared in physiological saline (scale 3 McFarland), and 0.1 mL of each suspension was plated onto Albicans ID2® plates. The reading of the plates and interpretation of the results were conducted after 24h, 48h and 72h of incubation at 32ºC. In order to prevent prejudice in reading the results, the determination of the color and the size of colonies on Albicans ID2® agar plates was performed by two different readers. Identification of yeasts was carried out according to the manufacturer's instruction: smooth blue colonies were identified as *Candida albicans* and other types of colonies were considered non-*albicans* yeasts.

Sensitivity, specificity, positive predictive value and negative predictive value were calculated comparing the identification of *C. albicans* on Albicans ID2® and the preliminary identification by standard methods (5).

A total of 330 isolates were successfully cultured on Albicans ID2®. Table 1 presents the colony color exhibited by the tested isolates. The plates were incubated for 72h, but 24h and 48h partial results were also recorded.

After 24h of incubation at 32ºC, all 100 *C. albicans* strains exhibited growth on the chromogenic media and were identified by their blue pigmentation on the media (sensitivity 100%). There was no significant change in the reading pattern of the blue colonies after 48h and 72h of incubation. *C. dubliniensis* strains also gave smooth blue colonies but, unlike *C. albicans* strains, exhibited weak growth at 24h reading. Significant growth of *C. dubliniensis* was observed only after 48h of incubation. This is an original finding because previous publications did not attempt to evaluate the dynamics of colony growth between 24h and 72h of incubation (4,7).

Table 1. Colony colors exhibited by 330 yeast isolates cultured on Albicans ID2® media after 48 h incubation at 35ºC.

Species	Number of strains		
	Blue	White	Total
C. albicans	100	0	100
C. tropicalis	3	47	50
C. rugosa	30	0	30
C. glabrata	0	30	30
C. dubliniensis	20	0	20
C. parapsilosis	0	20	20
C. lusitaniae	0	20	20
C. guilliermondii	0	20	20
C. krusei	0	20	20
Trichosporon spp.	5	5	10
C. neoformans	0	10	10
Total	158	172	330

Besides *C. albicans* and *C. dubliniensis* strains, isolates representative of other species also exhibited blue colonies on Albicans ID2® media after 48h of incubation, including 30 out of 30 *C. rugosa* (100%) strains, 3 out of 50 *C. tropicalis* (6%) and 5 out of 10 *Trichosporon* spp. (50%) strains.

Problems in the misidentification of pathogens by using the Candida ID2® media were also reported by other authors. Fricker-Hidalgo *et al.* (7) found that blue colonies were exhibited on Albicans ID2® cultures by 10 out of 10 *C. dubliniensis* strains (100%), 15 out of 29 *C. tropicalis* strains (51.7%), 22 out of 28 *Trichosporon* spp. strains (78.6%) and 2 out 5 *C. rugosa* (40%) strains. Cárdenes *et al.* (4) reported misidentification of yeast by using Candida ID2®, after testing *C. tropicalis* (2 out 69 or 2.8%), *C. parapsilosis* (3 out 76 or 3.9%) and *C. glabrata* (2 out 47 or 4%) strains. In our study, the identification of *C. albicans* using Candida ID2® presented specificity of 82% and predictive positive value of 72.4%, which are very similar to those obtained by Fricker-Hidalgo *et al.* (7).

Comparing the performance of Albicans ID® and Albicans ID2® in the identification of *C. albicans* strains (Table 2), both methods present some limitations in the identification of *C. dubliniensis*, *C. rugosa*, *C. tropicalis*, *Trichosporon* spp. *and Cryptococcus* spp.

In conclusion, Albicans ID2® chromogenic medium present some advantages in terms of the ease, rapidity and reliability for identification of *C. albicans*. However, this chromogenic medium has low specificity and low positive predictive value in the identification of *C. albicans* strains. Therefore, we do not recommend the use of Albicans ID2® as the only screening test for the identification of *C. albicans* from clinical specimens.

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RESUMO

Performance do meio cromogênico Albicans ID2® para a rápida identificação de *Candida albicans*

O objetivo do nosso estudo foi avaliar a eficácia do meio cromogênico Albicans ID2® (bioMérieux, France) na identificação de *Candida albicans* entre 330 amostras de leveduras. As cepas de *C. albicans* (100) e *C. dubliniensis* (20)

Table 2. Summary of studies evaluating the performance of Albicans ID® and Albicans ID2® plates for the rapid identification of *Candida albicans*.

Authors	Year	StrainsTested (n)	FP	FN	S %	SP%	PPV%	PNV%
Results with Albicans ID								
Lipperheide *et al.* (12)	1993	330 (186)	28	0	100	86.6	93	100
Willinger *et al.* (20)	1994	340 (247)	1	1	99.6	98.6	99.6	98.9
Rouselle *et al.* (16)	1994	723 (352)	5	22	93.8	98.6	98.5	94.3
De Champs *et al.* (6)	1995	320 (177)	3	7	96	97.9	98.3	95.2
Contreras *et al.* (5)	1996	250 (122)	0	1	99.2	100	100	99.2
Hoppe *et al.* (10)	1999	615 (281)	1	3	93	99	99	98
Godoy *et al.* (8)	2001	190 (80)	6	0	100	90	88	100
Results with Albicans ID2								
Fricker-Hidalgo *et al.* (7)	2001	247 (128)	46	0	100	82.7	73.5	100
Cárdenes *et al.* (4)	2002	423 (163)	9	0	98.2	96.6	94.7	98.8
Present study	2004	330 (100)	38	0	100	82	72.4	100

(n) number of *C. albicans* isolates; FP = false positive; FN = false negative; S = sensitivity; SP = specificity; PPV = predictive positive value; PNV = predictive negative value.

exibiram coloração azul quando semeadas em Albicans ID2®. Contudo, a coloração azul também foi verificada em culturas de *C. rugosa* (30/30) e *C. tropicalis* (3/50).

Palavras-chave: *Candida albicans*, meio cromogênico, meio Albicans ID2®

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