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Evaluation of Bichro-latex Albicans, a New Method for Rapid Identification of *Candida albicans*

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A method for identification of *Candida albicans* within 5 min was evaluated by using 4,643 yeast isolates. Six false-positive and three false-negative reactions were observed. The specificity (99.87%) and sensitivity (99.74%) obtained indicate that the Bichro-latex albicans test is a useful method for the rapid identification of *C. albicans* colonies.

The incidence of candida infections has increased progressively (6). These infections affect predominantly immunodeficient patients (leukemic, organ transplant, and human immunodeficiency virus-infected patients) or patients under predisposing conditions (extensive surgical procedures or prolonged antibacterial, cytotoxic, or immunosuppressive treatment, among others). *Candida albicans* is the most frequent species isolated from those patients, and therefore, methods for the rapid identification of this fungus are useful in the standard microbiology laboratories.

Traditionally, rapid identification of *C. albicans* depends on the germ tube test, which can identify *C. albicans* strains in 2 h since the fungus produces germ tubes when it grows at 37°C in serum. However, up to 5% of the *C. albicans* isolates have been reported as germ tube negative, and non-*C. albicans* isolates may produce some structures which can be misinterpreted as germ tubes (13). In the last decade, a number of systems based on the detection of enzyme activities have been evaluated for the rapid identification of *C. albicans* (3, 10). Bichro-latex albicans is a new method for identifying *C. albicans* isolates by latex particle agglutination using a monoclonal antibody specific for *C. albicans*. In this study, we have evaluated the capability of the Bichro-latex albicans to accurately identify *C. albicans* from reference strains and clinically isolated yeasts.

Reference strains used as controls in this study were obtained from the National Collection of Pathogenic Fungi, Bristol, England (NCPF), the Colección Española de Cultivos Tipo, Valencia, Spain (CECT), the American Type Culture Collection, Rockville, Md. (ATCC), Institut Pasteur, Paris, France (IP), the National Collection of Yeast Cultures, Norwich, England (NCYC), and the Central Bureau Voor Schimmel Cultures, Baarn, The Netherlands (CBS) and included *C. albicans* NCPF 3153, NCPF 3156, NCPF 3205, NCPF 3255, NCPF 3116, NCPF 3119, NCPF 3155, CECT 1687, ATCC 66396, ATCC 76615, ATCC 64548, ATCC 64550, ATCC 90028, ATCC 90029, ATCC 24433, ATCC 46167, ATCC 28367, ATCC 38696, ATCC 44831, IP 88465, and IP 88665; *Candida stellatoidea* NCPF 3108 and ATCC 20408; *Candida tropicalis* NCPF 3111, NCYC 1393, and ATCC 28707; *Candida* (*Torulopsis*) *glabrata* NCPF 3240, ATCC 90030, and CBS 859; *Candida krusei* NCPF 3100, NCPF 3321, and IP 208.52; *Candida parapsilosis* NCPF 3103, NCPF 3104, ATCC 22019, and ATCC 90018; *Candida guilliermondii* NCPF 3099 and IP 821.63; *Candida kefyr* IP 842.74 and ATCC 28838; *Candida viswanathii* NCPF 3151; and *Cryptococcus neoformans* NCPF 3170, ATCC 900112, and ATCC 900113. The rest of the yeasts used (4,599) were clinical isolates previously isolated at our laboratories. Clinical isolates were identified by their carbohydrate assimilation patterns on ID 32C strips (bioMérieux, Marcy-l'Etoile, France). The test strips were incubated for up to 48 h and evaluated spectrophotometrically with the ATB reader and corresponding identification software. The isolates are listed in Table 1. Five hundred and ten *C. albicans* isolates were also tested by the germ tube formation test in horse serum (8). The serotype for some *C. albicans* isolates was determined with the rabbit polyclonal Candida Check factor 6 (IF6) antiserum (Iatron Laboratories, Inc., Higashi-Kanda, Chiyoda, Tokyo, Japan), which was performed according to the manufacturer's instructions.

The 4,599 clinical isolates included 1,611 isolates which had been grown directly on Sabouraud glucose agar with chloramphenicol (bioMérieux) at 37°C for 24 to 72 h from clinical materials. The remaining *C. albicans* isolates had been isolated over the last few years. They were maintained at 4°C on slants containing 20 g of glucose, 10 g of yeast extract, and 20 g of agar per liter and subcultured every 3 to 4 months. For this study, they were subcultured onto Sabouraud glucose agar (bioMérieux) at 37°C for 24 h to check their viability. In one experiment, 113 isolates were also grown on Sabouraud glucose agar with chloramphenicol (bioMérieux), Sabouraud glucose agar with gentamicin (bioMérieux), 5% sheep blood Columbia agar (bioMérieux), and Chocolate agar (bioMérieux) at 37°C for 24 h. All yeasts were tested in a blind fashion, and results were compiled at the end of the study. Bichro-latex albicans (Fumouze Diagnostics, Asnières, France) consists of red latex particles coated with a monoclonal antibody (LIB 3H8). This monoclonal antibody presents a reactivity very similar to that shown by monoclonal antibody 1B12 (5, 14). It reacts with the protein moiety of a glycoprotein of >200 kDa, specifically expressed in the *C. albicans* cell wall. The monoclonal antibody was produced by standard methods, and it was purified from ascites fluid by chromatography on a DEAE

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TABLE 1. Identification of *C. albicans* by Bichro-latex albicans

Species	No. of isolates	
	Tested	Positive by Bichro-latex albicans
C. albicans	2,322	2,319
C. glabrata	1,059	3
C. tropicalis	328	0
C. parapsilosis	240	1
C. kefyr	186	1
C. krusei	132	0
C. guilliermondii	84	1
C. lusitaniae	41	0
C. famata	9	0
C. inconspicua	8	0
C. holmii	4	0
C. lipolytica	3	0
C. lambica	3	0
C. intermedia	3	0
C. stellatoidea	2	0
C. utilis	2	0
C. norvegensis	2	0
C. pelliculosa	2	0
C. silvicola	1	0
C. viswanathii	1	0
C. zeylanoides	1	0
Saccharomyces cerevisae	148	0
Cryptococcus neoformans	14	0
Trichosporon cutaneum	18	0
Trichosporon mucoides	4	0
Trichosporon capitatum	2	0
Hansenula anomala	10	0
Rodothorula rubra	7	0
Pichia sp.	1	0

trisacryl LS column (Sepracor). The latex particles are in suspension in a green dye, giving a brown mixture. For each isolate, three isolated colonies were emulsified in 20 μl of a dissociating agent, which contains enzymes to expose the antigen recognized by the monoclonal antibody, and the test was performed according to the manufacturer's instructions. The interpretation of agglutination was easy since positive isolates showed red agglutinates in a green background while negative isolates showed no agglutination, with the suspension keeping its homogeneous brown color. The sensitivity and specificity were determined as number of true positives/(number of true positives + number of false negatives) and number of true negatives/(number of true negatives + number of false positives), respectively.

Bichro-latex albicans correctly identified 2,319 of the 2,322 *C. albicans* isolates grown on Sabouraud dextrose agar. Six isolates (three *C. glabrata*, one *C. parapsilosis*, one *C. kefyr*, and one *C. guilliermondii*) of the 2,318 non-*C. albicans* yeast isolates showed a false-positive reaction. No differences in performance of the Bichro-latex albicans test were observed regarding the source of the isolates, since identification was correct both in colonies directly grown from clinical materials and in those subcultured on Sabouraud dextrose agar. The specificity and sensitivity obtained by the Bichro-latex albicans test were 99.87 and 99.74%, respectively. In one experiment, the influence of the growth medium on the expression of the epitope reacting with monoclonal antibody LIB 3H8 was studied in 113 yeast isolates (34 *C. albicans* and 79 non-*C. albicans*) grown on Sabouraud glucose agar with chloramphenicol, Sabouraud glucose agar with gentamicin, 5% sheep blood agar, and Chocolate agar. Irrespective of the growth medium, all *C. albicans* isolates studied gave a positive agglutination test and the non-*C. albicans* isolates studied showed no agglutination. In another experiment, 510 *C. albicans* isolates were tested by the Bichro-latex albicans test and the germ tube test. All the isolates were correctly identified by Bichro-latex albicans, while 22 isolates did not produce germ tubes (4.31%), a percentage similar to that found in other studies (3, 10). To determine whether the serotype of *C. albicans* had any influence on the performance of the Bichro-latex albicans test, 50 *C. albicans* serotype A and 50 *C. albicans* serotype B isolates were tested. In all cases, a positive reaction was observed.

The results of this study confirm those of Robert et al. (11) and Dromer et al. (4) regarding the accuracy of Bichro-latex albicans in identifying *C. albicans*. The test was easy to use, and the reaction was distinct and easy to interpret. Due to the colored latex beads, there was no need to check for autoagglutination, a problem observed in *C. albicans* agglutination assays (2). Identification of *C. albicans* by Bichro-latex albicans requires the previous isolation of the yeast on a primary isolation medium, which usually takes 24 to 48 h. This is also the case for other rapid identification systems, but Bichro-latex albicans has the advantage that it can be performed directly on colonies grown on a variety of primary isolation media and there is no need for a subculture in a special medium. If the isolation is carried out in one of the new chromogenic media which allow the simultaneous isolation and identification of *C. albicans* (1, 9, 12, 15), a further identification may not be necessary. However, if the isolation is carried out in a nonchromogenic medium, a situation which is likely to occur in most microbiology laboratories, Bichro-latex albicans is as quick as some methods based on the detection of enzymes (3), without needing any special equipment, and it is faster and more sensitive than the germ tube test. The usefulness of Bichro-latex albicans could become more widespread if the test is applied to the rapid identification of *C. albicans* directly from blood culture broths (7), since *C. albicans* is the first yeast species isolated from blood cultures (16).

In conclusion, the Bichro-latex albicans test was sensitive, specific, and easy to perform and allowed for the identification of *C. albicans* colonies taken directly from different isolation media.

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REFERENCES

1. **Baumgartner, C., A. M. Freydiere, and Y. Gille.** 1996. Direct identification and recognition of yeast species from clinical material by using Albicans ID and CHROMagar Candida plates. J. Clin. Microbiol. **34:**454–456.
2. **Brawner, D. L.** 1991. Comparison between methods for serotyping of *Candida albicans* produces discrepancies in results. J. Clin. Microbiol. **29:**1020–1025.
3. **Dealler, S. F.** 1991. *Candida albicans* colony identification in 5 minutes in a general microbiology laboratory. J. Clin. Microbiol. **29:**1081–1082.
4. **Dromer, F., O. Ronin, L. Improvisi, and B. Dupont.** 1996. Utilité et limites du Bichro-latex albicans® pour l'identification rapide de *Candida albicans*. J. Mycol. Méd. **6:**91–92.
5. **Elorza, M. V., A. Marcilla, R. Sanjuan, S. Mormeneo, and R. Sentandreu.** 1994. Incorporation of specific wall proteins during yeast and mycelial protoplast regeneration in *Candida albicans*. Arch. Microbiol. **161:**145–151.
6. **Fraser, V. J., M. Jones, J. Dunkel, S. Storfer, G. Medoff, and W. C. Dunagan.** 1992. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. Clin. Infect. Dis. **15:**415–421.
7. **Laurent, F., P. Cahen, and P. Honderlick.** 1996. Utilisation du réactif Bichrolatex albicans® pour l'identification rapide de *Candida albicans* dans les

flacons d'hemoculture: resultats préliminaires. J. Mycol. Méd. **6:**19–21.
8. **Mackenzie, D. W. R.** 1962. Serum germ tube identification of *Candida albicans*. J. Clin. Pathol. **15:**563–565.
9. **Odds, F., and R. Bernaerts.** 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **12:**1923–1929.
10. **Perry, J. L., G. R. Miller, and D. L. Carr.** 1990. Rapid colorimetric identification of *Candida albicans*. J. Clin. Microbiol. **28:**614–615.
11. **Robert, R., R. Sentandreu, C. Bernard, and J. M. Senet.** 1994. Evaluation du reactif Bichrolatex albicans pour l'identification rapide de colonies de *Candida albicans*. J. Mycol. Méd. **4:**226–229.
12. **Rouselle, P., A. M. Freydiere, P. Couillerot, H. Montclos, and Y. Gille.** 1994. Rapid identification of *Candida albicans* by using Albicans ID and Fluoroplate agar plates. J. Clin. Microbiol. **32:**3034–3036.
13. **Salkin, I. F., G. A. Land, N. J. Hurd, P. R. Goldson, and M. R. McGinnis.** 1987. Evaluation of YeastIdent and Uni-Yeast-Tek yeast identification systems. J. Clin. Microbiol. **25:**625–627.
14. **Sanjuan, R., J. Zueco, J. Perez, C. Peñarroja, and R. Sentandreu.** 1996. A comparative study of the incorporation of a 1,6 β glucan and an O glycosylated protein epitope into the cell wall of *Candida albicans*. Microbiology **142:**2255–2262.
15. **San-Millán, R., L. Ribacoba, J. Pontón, and G. Quindós.** 1996. Evaluation of a commercial medium for identification of *Candida* species. Eur. J. Clin. Microbiol. Infect. Dis. **15:**153–158.
16. **Telenti, A., J. M. Steckelberg, L. Stockman, R. S. Edson, and G. D. Roberts.** 1991. Quantitative blood cultures in candidemia. Mayo Clin. Proc. **66:**1120–1123.