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Rapid, Colorimetric Identification of *Candida albicans*

JACK L. PERRY,[1]* GLENDON R. MILLER,[2] AND DENNIS L. CARR[3]

Clinical Microbiology Laboratories, Veterans Administration Medical Center,[1] and St. Joseph Medical Center,[3] Wichita, Kansas 67218, and Department of Biological Sciences, The Wichita State University, Wichita, Kansas 67208[2]

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A total of 706 yeast isolates were evaluated in parallel by the *Candida albicans* Screen (CAS; Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) test and the germ tube (GT) test in comparison with the API 20C Yeast Identification System. The CAS and GT tests correctly identified 419 of the 422 isolates of *C. albicans* (99.3%). Two of the false-negative reactions occurring with the CAS were with GT-negative strains of *C. albicans*. There were two false-positive CAS reactions involving a single strain each of *C. parapsilosis* and *C. tropicalis*. Sensitivity and specificity for both tests exceeded 99%, with positive and negative predictive values of 99 and 98%, respectively.

Candidiasis is increasing as a complication in both immunocompromised and immunocompetent individuals. *Candida albicans* remains the single most frequently isolated yeast pathogen; however, *C. parapsilosis*, *C. tropicalis*, and *C. lusitaniae* have recently been reported as significant opportunistic pathogens (2–4, 6) colonizing patients and medical devices (5, 8, 11). With opportunistic yeasts isolated more frequently, there is an obvious need for rapid and cost-effective differentiation of *C. albicans* from other *Candida* species. The lengthy incubation necessary for biochemical characterization of clinical yeast isolates may hinder the clinical importance of yeast identification. Although the germ tube (GT) test is rapid (2 to 3 h), problems with GT-negative strains of *C. albicans* (9), misinterpretation of elongated blastoconidia as GTs, and time-consuming microscopy, coupled with the health hazards of handling pooled human sera (1), make this common test less desirable. Recent advances in rapid yeast identification include fluorogenic assays for differentiation of *C. parapsilosis* (10) and *C. albicans* (7).

We describe here a modification of a previously reported rapid test (7) involving the alteration of the test substrate and addition of a second substrate combined in one reaction tube and providing a colorimetric rather than fluorometric endpoint. The *Candida albicans* Screen (CAS; Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) was tested in parallel with the API 20C Yeast Identification System (Analytab Products, Plainview, N.Y.) and traditional GT test.

This 90-min test is based on colorimetric detection of two enzymes, L-proline aminopeptidase and β-galactosaminidase. Substrates *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide (NGL; 1 g/liter) and L-proline β-naphthylamide (PRO; 0.4 g/liter) were combined in one screw-cap tube (10 by 80 mm) containing 0.30 ml of liquid. A total of 706 clinical yeast isolates maintained on Sabouraud dextrose agar (SAB; Remel, Lenexa, Kans.) at room temperature not longer than 72 h prior to testing were used. Yeast colonies that were 24 to 72 h old, incubated at 30°C on SAB, were suspended in the substrate tube to achieve the density of a number 3 Wickerham standard, after which the tubes were incubated at 35 to 37°C for 90 min. Following incubation, one drop of 2% NaOH was added, mixed, and examined against a white background for the development of a distinct yellow indicating a positive NGL reaction. Results were recorded, and then two drops of cinnamaldehyde reagent (*p*-dimethylaminocinnamaldehyde; 0.1 g/liter) were added to the same tube and allowed to stand for 1 min without mixing. The NGL test must be performed before the PRO reaction. A positive PRO test was indicated by development of a pink to red at the top of the liquid. A positive reaction for both NGL and PRO was considered presumptive for the identification of *C. albicans*. Isolates tested from sheep blood and chocolate agars, as well as SAB with chloramphenicol and cycloheximide (Remel), SAB with chloramphenicol (Remel), and brain heart infusion with sheep blood, chloramphenicol, and gentamicin (Remel), produced results equivalent to those from SAB.

Results obtained with the GT and CAS tests compared with reference method identification and the distribution of organisms tested are summarized in Table 1. The CAS and GT tests both correctly identified 419 of the 422 *C. albicans* isolates tested. Two of the three CAS false-negative reactions were due to GT-negative strains of *C. albicans*. The sensitivity and specificity for the CAS exceeded 99%, and

TABLE 1. Comparison of the CAS and GT tests with API 20C identification

Organism (no. tested)	No. (%) positive by:	
	CAS	GT
Candida albicans (422)	419 (99.3)	419 (99.3)
Torulopsis glabrata (125)	0	0
Candida krusei (3)	0	0
Candida lambica (5)	0	0
Candida lipolytica (7)	0	0
Candida lusitaniae (4)	0	0
Candida parapsilosis (40)	1 (2.5)	0
Candida pseudotropicalis (1)	0	0
Candida rugosa (1)	0	0
Candida stellatoidea (3)	0	3 (100)
Candida tropicalis (77)	1 (1.3)	0
Cryptococcus laurentii (1)	0	0
Cryptococcus neoformans (1)	0	0
Hansenula polymorpha (1)	0	0
Pichia anomala (1)	0	0
Rhodotorula rubra (2)	0	0
Saccharomyces cerevisiae (6)	0	0
Trichosporon beigelii (6)	0	0

* Corresponding author.

positive and negative predictive values were 99 and 98%, respectively. Immature colonies of *C. albicans* did not produce strong reactions, whereas after 18 to 24 h of incubation, strong reactions were evident and remained detectable from 6- to 8-week-old cultures kept on SAB at room temperature.

The CAS is conveniently packaged in a 48-test kit complete with reagents and has a 6-month shelf life. Compared with the GT test, the CAS was much easier to interpret because it has a nonmicroscopic, colorimetric endpoint.

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