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Comparison of the MUREX *C. albicans*, Albicans-Sure, and BactiCard Candida Test Kits with the Germ Tube Test for Presumptive Identification of *Candida albicans*

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The MUREX *C. albicans* (MC) (Murex Diagnostics), Albicans-Sure (AS) (Clinical Standards Laboratories), and BactiCard Candida (BC) (Remel) test kits were compared with the germ tube (GT) test for the rapid, presumptive identification of *Candida albicans*. All three test kits detect the enzymes L-proline aminopeptidase and β-galactosaminidase in yeast cells grown on culture media and are based on the principle that *C. albicans* produces both enzymes whereas other yeasts produce only one or neither of the enzymes. The organisms evaluated were fresh clinical isolates identified by methods routinely used in our laboratory (API 20C system and conventional methods) and included 303 *C. albicans* isolates, 153 *Candida glabrata* isolates, 70 *Candida tropicalis* isolates, 36 *Candida parapsilosis* isolates, 13 isolates of other *Candida* spp., 5 *Cryptococcus neoformans* isolates, and 3 *Saccharomyces cerevisiae* isolates. The MC, AS, BC, and GT tests detected 299 (98.7%), 300 (99.0%), 301 (99.3%), and 287 (94.7%) *C. albicans* isolates, respectively. There was one false-positive result with both the MC and BC kits and two false-positive results with the GT test. The enzymatic methods evaluated in this study provide rapid and accurate alternatives to the GT test for the presumptive identification of *C. albicans*.

The number of fungal infections caused by yeasts, particularly among immunocompromised or immunosuppressed patients, has dramatically increased over the past several decades. *Candida albicans*, the most frequent cause of candidiasis, can account for up to 75% of the yeasts recovered from clinical specimens (10). With *C. albicans* accounting for such a large percentage of yeasts isolated, any test which will rapidly and cost effectively differentiate *C. albicans* from other *Candida* spp. is desirable. For over 3 decades the germ tube (GT) test (1, 4, 10), which is based on the fact that *C. albicans* produces short, slender, tube-like structures (germ tubes) when incubated at 35 to 37°C for 2 to 4 h in pooled human, rabbit, or sheep serum, has served this purpose. Although very reliable, the GT test is not without problems. Up to 5% of the strains of *C. albicans* may be germ tube negative, and false-positive results can occur with *Candida stellatoidea* (now classified as *C. albicans*) and other yeasts that produce germ tube-like structures, e.g., pseudohyphae (5, 8). These limitations, in addition to the need for time-consuming microscopy, have spawned research into developing suitable alternatives. Several investigators have demonstrated that *C. albicans* can be rapidly and accurately identified by using fluorogenic (2, 5, 9) and colorimetric (2, 6) enzyme substrates. Test kits, utilizing these substrates, have recently become commercially available. This study compares three of these commercially available tests and the GT test for the rapid, presumptive identification of *C. albicans.*

A total of 583 isolates, which consisted of 11 different species of yeasts, were analyzed in this study. These organisms were fresh clinical isolates, 445 of which were cultured from specimens received in the Microbiology Laboratory, Polyclinic Medical Center, Harrisburg, Pa., and 138 of which were provided courtesy of Tasna Kitch, Milton S. Hershey Medical Center, Hershey, Pa. (87 isolates), and Robert L. Sautter, Harrisburg Hospital, Harrisburg, Pa. (51 isolates). The isolates tested were from a variety of sources which included urine (214 specimens), respiratory tracts (176 specimens), wounds (53 specimens), stools (44 specimens), blood (23 specimens), spinal fluid (2 specimens), other body fluids (12 specimens), and other sources not stated (59 specimens). Isolates either were tested from primary isolation media, e.g., sheep blood agar or chocolate agar, or were subcultured onto Sabouraud dextrose agar at room temperature before being tested. All tests were performed on cultures at least 24 h old but less than 96 h old.

Yeast-like colonies growing on primary isolation media or Sabouraud dextrose agar were routinely Gram stained and if found to be yeast cells microscopically were tested for germ tube production (1) and were identified with the API 20C system (bioMerieux Vitek, Inc., Hazelwood, Mo.) (3). Isolates for which the API 20C gave a questionable identification or one of low selectivity were subjected to supplemental testing as recommended by the manufacturer or, if needed, by additional conventional methods (7, 10).

The commercially available rapid enzymatic tests were performed according to the manufacturers' instructions and are described below. The MUREX *C. albicans* (MC) (Murex Diagnostics, Inc., Norcross, Ga.) test was performed by placing a disc containing the substrates *p*-nitrophenyl-*N*-acetyl-β-D-galactosamide (NGL) and L-proline-β-naphthylamide (PRO) into one of the test tubes provided with the kit. One drop (approximately 10 μl) of distilled or deionized water is added to moisten the disc. With a plastic inoculating loop, a heavy, visible "paste" of yeast cells is removed from colonies growing on culture medium and then used to inoculate the disc. The tube is then capped and incubated at 35 to 37°C for 30 min. After incubation, 1 drop of 0.3% sodium hydroxide reagent is added to the tube. If the enzyme β-galactosaminidase is present, the substrate NGL will be hydrolyzed and a yellow color will develop because of the liberation of *p*-nitrophenol. A

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TABLE 1. Comparison of MC, AS, BC, and GT tests with API 20C identification system

Organism (no. of isolates tested)	No. (%) positive by:			
	MC	AS	BC	GT
Candida albicans (303)	299 (98.7)	300 (99.0)	301 (99.3)	287 (94.7)
Candida glabrata (153)	0	0	1 (<1)	2 (<1)
Candida humicola (1)	1 (100)	0	0	0
Candida tropicalis (70)	0	0	0	0
Candida parapsilosis (36)	0	0	0	0
Candida lusitaniae (6)	0	0	0	0
Candida krusei (4)	0	0	0	0
Candida kefyr (1)	0	0	0	0
Candida stellatoidea (1)	0	0	0	0
Cryptococcus neoformans (5)	0	0	0	0
Saccharomyces cerevisiae (3)	0	0	0	0

second reagent, 0.01% *p*-dimethylaminocinnamaldehyde, is then added to the tube. The development of a pink to red color within 1 min indicates hydrolysis of the substrate PRO by the enzyme L-proline aminopeptidase. *C. albicans* is positive for both enzymes, while other yeasts may be positive for one or the other enzyme but not both.

The Albicans-Sure (AS) (Clinical Standards Laboratories, Inc., Rancho Dominguez, Calif.) test card contains two circles designated A (blue circle) and B (red circle). Circle A contains the substrate 4-methylumbelliferyl-*N*-acetyl-β-galactosamine (NAG), and circle B contains proline-*p*-nitroanilide (PROL). The substrates are rehydrated by adding 10 μl of distilled or deionized water to each circle. Each circle is inoculated with 2 or 3 colonies of the test isolate by using a sterile plastic inoculating loop. The test card is then incubated for 5 min at room temperature, after which 1 drop of phosphate buffer (pH 10) is added to circle A and 1 drop of *p*-dimethylaminocinnamaldehyde reagent is added to circle B. If the enzyme β-galactosaminidase is present, the substrate NAG (circle A) will be cleaved, releasing the highly fluorescent compound 4-methylumbelliferone (blue-white fluorescence), which is detected after 30 s by using a hand-held long-wave UV light. If the enzyme L-proline aminopeptidase is also present, it will cleave the substrate PROL (circle B), forming a blue precipitate. *C. albicans* is positive for both enzymes, while other yeasts may be positive for one or the other enzyme but not both.

The BactiCard Candida (BC) (Remel, Lenexa, Kans.) test card consists of two separate test circles containing the substrates 4-methylumbelliferyl-*N*-acetyl-β-D-galactosaminide (MUGAL) and PRO. The substrates are rehydrated by adding 1 drop of BC rehydrating fluid to each test circle, being careful not to oversaturate the test area. With an applicator stick, a heavy, visible paste of yeast cells is removed from colonies growing on culture medium and used to inoculate the test circles. The test card is then incubated for 5 min at room temperature, after which time 1 drop of BC color developer is added to the PRO test circle. If the enzyme L-proline aminopeptidase is present, it will hydrolyze the substrate PRO, causing a red color to develop within 30 s. One drop of BC MUGAL reagent is then added to the MUGAL test circle. If the enzyme β-galactosaminidase is present, the substrate MUGAL will be cleaved, releasing the highly fluorescent compound 4-methylumbelliferone (bright blue fluorescence), which is detected by using a hand-held long-wave UV light. *C. albicans* is positive for both enzymes, while other yeast species may be positive for one or the other enzyme but not both.

Of the 583 isolates tested, 303 were identified by the API 20C system and conventional methods as *C. albicans*. Of the 303 *C. albicans* isolates tested, the MC, AS, BC, and GT kits identified 299, 300, 301, and 287, respectively (Table 1). Very few organisms were misidentified as *C. albicans*. There was one *Candida humicola* isolate misidentified by the MC kit, one *Candida glabrata* isolate misidentified by the BC kit, and two *C. glabrata* isolates misidentified by the GT test. Although *C. glabrata* does not produce germ tube-like structures, e.g., pseudohyphae, and therefore should not be misidentified with the GT test, other investigators (2) have reported similar results when the GT test is performed in a general microbiology laboratory. Sensitivity, specificity, and predictive values for the four methods are presented in Table 2. The three kits using rapid enzymatic methods had sensitivities greater than 98.0% and were more sensitive than the GT test, which had a sensitivity of 94.7%. The 5.3% false-negative rate for the GT test obtained in this study is consistent with that previously reported by others (8). The specificities and positive and negative predictive values for all three rapid enzymatic tests were greater than 98.6%. An analysis of discrepant results is presented in Table 3. All three rapid enzymatic methods failed to

TABLE 2. Comparative analysis of the four screening methods for *C. albicans*

Screening test	Sensitivity (%)	Specificity (%)	Predictive value (%) of result:	
			Positive	Negative
MC	98.7	99.6	99.6	98.6
AS	99.0	100.0	100.0	98.9
BC	99.3	99.6	99.6	99.3
GT	94.7	99.3	99.3	94.5

TABLE 3. Analysis of discrepant results for the three rapid enzymatic methods[a]

Organism	Laboratory identification	MC		AS		BC	
		NGL	PRO	NAG	PROL	MUGAL	PRO
C. albicans	33	−	−	+	−	+	+
	106	−	+	+	+	+	+
	161	+	−	+	−	+	−
	321	+	−	+	−	+	−
C. glabrata	462	−	−	−	−	+	+
C. humicola	173	+	+	−	−	−	−

[a] −, negative result; +, positive result.

TABLE 4. Cost comparison of methods

Test method	Kit size[a]	Cost per test ($)[b]
GT		0.46
AS	25	1.06
	50	0.94
MC	50	1.26
BC	25	1.76

[a] Number of tests per kit.

[b] The cost per test for the AS, MC, and BC kits was based on the list price of the kit. The cost per test for the GT test was based on the list price for the following reagents and supplies: Trypticase soy broth, rabbit coagulase plasma, pipettes, microscope slides, coverslips, and test tubes.

detect the enzyme L-proline aminopeptidase in the same two isolates (laboratory no. 161 and 321). Table 4 presents a comparison of costs of the three rapid enzymatic tests and the GT test. On the basis of the list price, the AS kit is the least expensive of the enzymatic tests and the BC kit is the most expensive. All three rapid enzymatic tests are more expensive than our in-house GT test. In conclusion, the MC, AS, and BC kits are more sensitive than and as specific as the GT test for presumptively identifying *C. albicans* isolated from clinical specimens. Reagent costs for the MC, AS, and BC kits are higher than those for the GT test; however, one must weigh the increased reagent costs against the advantages of a shorter incubation time (5 min for AS and BC and 30 min for MC compared with 2 to 4 h for GT), macroscopic reading of results, and elimination of the use of pooled human, rabbit, or sheep sera.

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REFERENCES

1. **Berardinelli, S., and D. J. Opheim.** 1985. New germ tube induction medium for the identification of *Candida albicans*. J. Clin. Microbiol. **22:**861–862.
2. **Dealler, S. F.** 1991. *Candida albicans* colony identification in 5 minutes in a general microbiology laboratory. J. Clin. Microbiol. **29:**1081–1082.
3. **Land, G. A., B. A. Harrison, K. L. Hulme, B. H. Cooper, and J. C. Byrd.** 1979. Evaluation of the new API 20C strip for yeast identification against a conventional method. J. Clin. Microbiol. **10:**357–364.
4. **MacKenzie, D. W. R.** 1962. Serum tube identification of *Candida albicans*. J. Clin. Pathol. **15:**563–565.
5. **Perry, J. L., and G. R. Miller.** 1987. Umbelliferyl-labeled galactosaminide as an aid in identification of *Candida albicans*. J. Clin. Microbiol. **25:**2424–2425.
6. **Perry, J. L., G. R. Miller, and D. L. Carr.** 1990. Rapid, colorimetric identification of *Candida albicans*. J. Clin. Microbiol. **28:**614–615.
7. **Roberts, G. D.** 1994. Laboratory methods in basic mycology, p. 752–758. *In* E. J. Baron, L. R. Peterson, and S. M. Finegold (ed.), Bailey & Scott's diagnostic microbiology, 9th ed. Mosby, St. Louis.
8. **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:**624–627.
9. **Smitka, C. M., and S. G. Jackson.** 1989. Rapid fluorogenic assay for differentiation of the *Candida parapsilosis* group and other *Candida* spp. J. Clin. Microbiol. **27:**203–206.
10. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723–737. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.