JOURNAL OF CLINICAL MICROBIOLOGY, May 2008, p. 1824–1825 Vol. 46, No. 5
0095-1137/08/$08.00+0 doi:10.1128/JCM.02350-07

Evaluation of the New Micronaut-*Candida* System Compared to the API ID32C Method for Yeast Identification▽

Z. Szabó,[1,2] B. Tóth,[2] M. Kovács,[3] G. Kardos,[2] A. Maráz,[3] F. Rozgonyi,[1]† and L. Majoros[2]*

Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary[1]; Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary[2]; and Department of Microbiology and Biotechnology, Faculty of Food Science, Corvinus University of Budapest, Budapest, Hungary[3]

Received 7 December 2007/Returned for modification 14 January 2008/Accepted 21 February 2008

A new system, Micronaut-*Candida*, was compared to API ID32C to identify 264 yeast (*Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. inconspicua*, *C. norvegensis*, *C. lusitaniae*, *C. guilliermondii*, *C. dubliniensis*, *C. pulcherrima*, *C. famata*, *C. rugosa*, *C. glabrata*, *C. kefyr*, *C. lipolytica*, *C. catenulata*, *C. neoformans*, *Geotrichum* and *Trichosporon* species, *Rhodotorula glutinis*, and *Saccharomyces cerevisiae*) clinical isolates. Results were in concordance in 244 cases. Eighteen out of the 20 of discordant results were correctly identified by Micronaut-*Candida* but not by API ID32C, as confirmed by PCR ribotyping.

The frequency of invasive fungal infections has increased dramatically during the past 3 decades (10). Correct identification is important for accurate therapy, as some fungi possess intrinsic resistance to certain antifungals (4). Conventional methods are time-consuming, while DNA-based methods are not available to all laboratories. Relatively simple tests based on assimilation reactions (API ID32C, API 20C, API *Candida*) are commercially available, though they may sometimes yield equivocal results or even lead to misidentification (1, 3, 5, 11).

A new microplate-based system with four tests on a single plate, Micronaut-*Candida* (Merlin Diagnostika GmbH, Bornheim, Germany), has been developed to identify medically important yeasts. Its database contains 31 species of six genera. A test contains 21 biochemical reactions and three controls (8 chromogenic substrates [*N*-acetyl-β-D-galactosaminidase, α-galactosidase, L-prolinaminopeptidase, *p*-nitrophenyl-β-glucoronidase, L-phenylalaninaminopeptidase, α-glucosidase, and β-glucosidase plus control], 14 carbohydrate assimilation tests [melibiose, D-xylose, L-rhamnose, gentibiose, D-glucose, inositol, cellobiose, saccharose, trehalose, galactose, maltose, lactose, raffinose, and assimilation control], and urease test with its control). After 24 h, results are read and interpreted automatically using the Micronaut Skan device and the Micronaut software.

(This work was presented in part at the 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 2006 [13a].)

The aim of our study was to identify 264 yeast clinical isolates with Micronaut-*Candida* and with API ID32C (BioMerieux, Marcy l'Etoile, France). We also tested 13 ATCC strains (*Candida albicans* 14053, *C. albicans* 10231, *C. parapsilosis* 22019, *C. tropicalis* 750, *C. krusei* 6258, *C. inconspicua* 16783, *C. norvegensis* 22977, *C. lusitaniae* 38533, *C. guilliermondii* 6260, *C. dubliniensis* CD36, *C. pulcherrima* 18406, *C. famata* 36239, and *C. rugosa* 2142). The majority of isolates were isolated from throat, sputum, blood, wound, urine, and vagina from in- and outpatients between November 2005 and April 2006. All *C. parapsilosis* isolates belonged to *C. parapsilosis* sensu stricto, determined as described earlier (13). *Candida dubliniensis* isolates were derived from a previous study (12). Some less common yeast species (*Geotrichum* and *Trichosporon* species) were from our collection.

Yeasts grown on Sabouraud dextrose agar were tested simultaneously with API ID32C and Micronaut-*Candida* according to the manufacturers' instructions. API ID32C and Micronaut-*Candida* were read after 48 and 24 h, respectively. In case of discrepancy, both methods were repeated. If these were in accord, we accepted them as final. Otherwise or when additional tests (microscopic morphology, esculin hydrolysis, growth at 45°C, nitrate assimilation) yielded equivocal results, PCR ribotyping was performed in each case and ribotyping results were accepted as valid identification (5).

The proportion of misidentifications was determined for each test and the relative accuracies of results were compared using Fisher's exact test.

API ID32C correctly identified all tested ATCC strains. Micronaut-*Candida* misidentified *C. norvegensis* and *C. rugosa* ATCC strains, both as *C. valida* according to the additional test proposed (microscopic morphology). As *C. pulcherrima* is not included in the Micronaut database, Micronaut could not identify the *C. pulcherrima* ATCC strain. Other ATCC strains were correctly identified.

Results obtained by both methods without additional tests were in concordance for *C. albicans* ($n = 40$), *C. glabrata* ($n = 40$), *C. parapsilosis* ($n = 35$), *C. tropicalis* ($n = 35$), *C. krusei* ($n = 35$), *C. kefyr* ($n = 10$), *C. dubliniensis* ($n = 9$), *C. guilliermondii* ($n = 7$), *C. lusitaniae* ($n = 7$), *C. lipolytica* ($n = 4$), *S. cerevisiae*, ($n = 8$), *C. famata* ($n = 2$), *C. catenulata* ($n = 2$), *C. rugosa* ($n = 1$), *C. neoformans* ($n = 2$), and *Geotrichum* ($n = 2$) and *Trichosporon* ($n = 2$) species isolates. In the cases of one of two *C. rugosa* and two of two *Rhodotorula glutinis* isolates, Micronaut-*Candida* needed additional tests (microscopic morphology and nitrate assimilation, respectively) to accord with API ID32C.

* Corresponding author. Mailing address: Dept. of Medical Microbiology, University of Debrecen, 4032 Debrecen, Nagyerdei krt. 98, Hungary. Phone: 52-411-717. Fax: 52-417-565. E-mail: major@med.unideb.hu.

† Present address: Microbiology Laboratory, Department of Dermatology, Dermatooncology, and Venerology, Semmelweis University, Budapest, Hungary.

▽ Published ahead of print on 5 March 2008.

API ID32C using an extra test (esculin hydrolysis) misidentified all tested *C. inconspicua* strains ($n = 12$) as *C. norvegensis* strains, as confirmed by PCR ribotyping (6). These strains were correctly identified with Micronaut-*Candida* using an additional test (microscopic morphology). Five *C. lusitaniae* strains were misidentified as *C. famata* and a single *C. tropicalis* strain was misidentified as *C. humicolus* with API ID32C but not with Micronaut-*Candida*. Two *C. pulcherrima* clinical isolates were correctly identified with API ID32C; Micronaut-*Candida* did not identify these strains, as *C. pulcherrima* is not included in the Micronaut database. These identities were also confirmed by PCR ribotyping.

Micronaut-*Candida* correctly identified *Candida* species occurring frequently in clinical practice (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*) within 24 h. Additionally, *Candida* species possessing primary (*C. krusei*) or secondary (*C. glabrata*) resistance to fluconazole (6, 9) were also identified accurately within 1 workday. Both methods correctly identified all tested *C. dubliniensis* isolates without additional tests. As *C. dubliniensis*, similarly to *C. glabrata*, may acquire fluconazole resistance readily during treatment (7), correct differentiation of this species from *C. albicans* within 24 h can be important.

API ID32C but not Micronaut-*Candida* misidentified 5 of 12 *C. lusitaniae* strains as *C. famata* strains. This may be problematic in clinical situations, as *C. famata*, in contrast to *C. lusitaniae*, often shows high MICs to triazoles and caspofungin (2, 8), while *C. lusitaniae* may be resistant to amphotericin B (4).

Micronaut-*Candida* never misidentified *C. inconspicua*, but an extra test (microscopic morphology) and extra days were needed to properly identify this inherently fluconazole-resistant species (5). Thus, fast and correct identification of *C. inconspicua* remains unresolved.

In summary, only 2 clinical isolates were unidentified/misidentified by Micronaut-*Candida*, while 18 were unidentified/misidentified by API ID32C (0.76% versus 6.82%; $P < 0.001$).

Micronaut-*Candida* is an easy-to-perform method to identify the most common *Candida* species within 24 h. Micronaut-*Candida* without extra tests identified 241 (91.3%) yeast isolates concordantly to API ID32C. In case of the 18 discordant isolates, ribotyping confirmed the Micronaut-*Candida* results.

Rare species with proven decreased susceptibility to certain antifungals (2, 5, 8, 10) were identified correctly by Micronaut-*Candida* with (*C. inconspicua*) or without extra tests (*C. kefyr*, *C. guilliermondii*, and *C. lusitaniae*). Thus, this new system seems to be a reliable and useful method for identification of medically relevant yeasts in routine mycology laboratories.

We thank Ferenc Somogyvári for providing *C. dubliniensis* isolates. Micronaut-*Candida* tests were provided by Merlin Diagnostika GmbH.

This study is part of the 8/3 PhD program of Semmelweis University, Budapest, Hungary, and was partly supported by grants OTKA T046186 and F048410.

REFERENCES

1. **Bernal, S., E. Martin-Mazuelos, M. Chavez, J. Coronilla, and A. Valverde.** 1998. Evaluation of the new API Candida system for identification of the most clinically important yeast species. Diagn. Microbiol. Infect. Dis. **32:** 217–221.
2. **Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado, M. J. Buitrago, A. Monzon, and J. L. Rodriguez-Tudela.** 2006. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. Antimicrob. Agents Chemother. **50:**917–921.
3. **Fricker-Hidalgo, H., O. Vandapel, M. A. Duchesne, M. A. Mazoyer, D. Monget, B. Lardy, B. Lebeau, J. Freney, P. Ambroise-Thomas, and R. Grillot.** 1996. Comparison of the new API Candida system to the ID 32C system for identification of clinically important yeast species. J. Clin. Microbiol. **34:**1846–18484.
4. **Kontoyiannis, D. P., and R. E. Lewis.** 2002. Antifungal drug resistance of pathogenic fungi. Lancet **359:**1135–1144.
5. **Majoros, L., G. Kardos, Á. Belák, A. Maráz, L. Asztalos, E. Csánky, Z. Barta, and B. Szabó.** 2003. Restriction enzyme analysis of ribosomal DNA shows that *Candida inconspicua* clinical isolates can be misidentified as *Candida norvegensis* with traditional diagnostic procedures. J. Clin. Microbiol. **41:**5250–5253.
6. **Orozco, A. S., L. M. Higginbotham, C. A. Hitchcock, T. Parkinson, D. Falconer, A. S. Ibrahim, M. A. Ghannoum, and S. G. Filler.** 1998. Mechanism of fluconazole resistance in *Candida krusei*. Antimicrob. Agents Chemother. **42:**2645–2649.
7. **Perea, S., J. L. Lopez-Ribot, B. L. Wickes, W. R. Kirkpatrick, O. P. Dib, S. P. Bachmann, S. M. Keller, M. Martinez, and T. F. Patterson.** 2002. Molecular mechanisms of fluconazole resistance in *Candida dubliniensis* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. Antimicrob. Agents Chemother. **46:**1695–1703.
8. **Pfaller, M. A., D. J. Diekema, S. A. Messer, L. Boyken, R. J. Hollis, and R. N. Jones.** 2004. In vitro susceptibilities of rare *Candida* bloodstream isolates to ravuconazole and three comparative antifungal agents. Diagn. Microbiol. Infect. Dis. **48:**101–105.
9. **Pfaller, M. A., S. A. Messer, L. Boyken, S. Tendolkar, R. J. Hollis, and D. J. Diekema.** 2004. Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. J. Clin. Microbiol. **42:**3142–3146.
10. **Pfaller, M. A., and D. J. Diekema.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20:**133–163.
11. **Ramani, R., S. Gromadzki, D. H. Pincus, I. F. Salkin, and V. Chaturvedi.** 1998. Efficacy of API 20C and ID 32C systems for identification of common and rare clinical yeast isolates. J. Clin. Microbiol. **36:**3396–3398.
12. **Somogyvari, F., I. Doczi, J. Serly, S. Ahmad, and E. Nagy.** 2007. Rapid discrimination between *Candida albicans* and *Candida dubliniensis* by using real-time polymerase chain reaction. Diagn. Microbiol. Infect. Dis. **58:**367–369.
13. **Tavanti, A., A. D. Davidson, N. A. R. Gow, M. C. J. Maiden, and F. C. Odds.** 2005. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. J. Clin. Microbiol. **43:**284–292.

13a. **Tóth, B., E. Fulusi, C. Miszti, Á. Borbély, G. Kardos, and L. Majoros.** 2006. Abstr. 16th Congress Int. Soc. Hum. Anim. Mycol., Paris, France, 25 to 29 June 2006, abstr. P-0428.