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Note

Evaluation of Bichro-Dubli Fumouze® to distinguish *Candida dubliniensis* from *Candida albicans*

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Abstract

We have evaluated the ability of the Bichro-Dubli Fumouze® (Fumouze Diagnostics, Levallois-Perret, France) latex agglutination test to identify colonies of *Candida dubliniensis* grown on different media. The test was positive for 103 of 106 isolates of *C. dubliniensis* and negative for *Candida albicans* and other *Candida* species studied. The sensitivity and specificity of the test were 97.1% and 100%, respectively. The test is very rapid, simple, and reliable giving the same results independently of whether the colonies are grown previously on Sabouraud dextrose agar, CHROMagar Candida medium, Candida ID2 medium, or CHROMagar–Pal's medium.

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Candida dubliniensis is a recently identified opportunistic yeast (Sullivan et al., 1995) that shares several phenotypic characteristics with *Candida albicans*, but they are genetically distinguishable. *C. dubliniensis* was primarily recovered from the oral cavities of HIV-infected and AIDS patients (Sullivan et al., 1997). More recently, it has also been recovered from urinary, vaginal, respiratory, and fecal specimens from cases of systemic disease in HIV- and non–HIV-infected patients (Meis et al., 1999; Pinjon et al., 1998).

There are few reported cases of candidemia due to *C. dubliniensis* in the world in HIV-infected patients, chemotherapy-induced immunosuppression, and bone marrow transplantation patients (Boyle et al., 2002; Meis et al., 1999; Salesa et al., 2001). Although many reports indicate that most strains of *C. dubliniensis* are susceptible to antifungal agents (Pfaller et al., 1999; Quindós et al., 2004), fluconazole-resistant strains have been detected and they are easily derived in vitro (Moran et al., 1998).

There are a limited number of phenotypic tests that can differentiate between *C. albicans* and *C. dubliniensis*. The ability to grow at 42–45 °C (Pinjon et al., 1998); the different colony color development in chromogenic media such as CHROMagar Candida medium (Kirkpatrick et al., 1998), Candida ID medium (Quindós et al., 2001), or Candida ID2 medium (Eraso et al., 2005); chlamydospores production in different media (Al Mosaid et al., 2003; Mosca et al., 2003; Sahand et al., 2005); differential carbohydrate assimilation (Cárdenes-Perera et al., 2004; Pincus et al., 1999); and reactivity with a specific anti-*C. dubliniensis* serum (Bikandi et al., 1998) have all been proposed as useful phenotypic tests. However, molecular techniques remain the most reliable method for *C. dubliniensis* identification (Sullivan et al., 1995), but its availability in many microbiologic laboratories is problematic. Bichro-Dubli Fumouze® (Fumouze Diagnostics, France) is a recently commercialized method for identifying *C. dubliniensis* isolates by latex agglutination with particles coated with a monoclonal antibody (12F7-F2) which allows the specific detection of an antigen located on the surface of *C. dubliniensis* blastoconidia.

In this study, we have evaluated the ability of Bichro-Dubli Fumouze® (Fumouze Diagnostics) to differentiate *C. dubliniensis* from *C. albicans* and other *Candida* species.

Reference strains were obtained from the National Collection of Pathogenic Fungi (NCPF), Bristol, UK; the Centraalbureau voor Schimmelcultures (CBS), Baarn,

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Netherlands; the American Type Culture Collection (ATCC), Manassas, VA; and the Colección Española de Cultivos Tipo (CECT), Valencia, Spain, and included *C. dubliniensis* NCPF 3949, CBS 2747, CBS 8500, CBS 8501, and CECT 11473; *C. albicans* serotype A NCPF 3153 and *C. albicans* serotype B NCPF 3156; *Candida tropicalis* NCPF 3111; *Candida glabrata* NCPF 3203; *Candida krusei* ATCC 6258; *Candida guilliermondii* NCPF 3099; *Candida parapsilosis* ATCC 22019; *Candida lusitaniae* ATCC 64125; and *Candida kefyr* ATCC 28838.

Clinical isolates from different anatomic sites (oral, vaginal, sputum, and blood) included 101 isolates of *C. dubliniensis* (87 genotype 1, 8 genotype 2, 1 genotype 3, and 5 genotype 4) and 28 isolates of *C. albicans*. All these isolates were identified by their carbohydrate assimilation patterns in API ID 32C (bioMérieux, Marcy-l'Etoile, France) and their identity was confirmed by polymerase chain reaction (PCR) with specific primers based on the genomic sequences of DNA topoisomerase II (Kanbe et al., 2002). The 4 genotypes of *C. dubliniensis* were assigned according to the method of Gee et al. (Brena et al., 2003; Gee et al., 2002).

Each isolate was grown simultaneously on Sabouraud dextrose agar (SDA, Difco; Becton, Dickinson and Co., Le Pont-De-Claix, France), CHROMagar Candida medium (CHA, CHROMagar, Paris, France), Candida ID2 medium (CAID2, bioMérieux), and CHROMagar–Pal's medium (CH-P, Sahand et al., 2005). After 48 h at 37 °C (SDA, CHA, and CAID2) or 30 °C (CH-P), 2–3 colonies were emulsified in 20 μL of the homogenized latex reagent with the disposable stirrers, and the results were obtained between 3 and 5 min. In case of positive reaction the agglutinated latex beads moved progressively toward the periphery, forming a blue edge around a central pink field, whereas in the negative reaction—no agglutination—the reagent kept its original purple appearance (Fig. 1).

Of 106 isolates of *C. dubliniensis*, 103 (97.1%) gave a positive reaction with the Bichro-Dubli Fumouze®, 4 of them were weakly positive (the 4 were from oral origin and genotype 4), and the remaining 3 isolates were negative (Table 1). The 3 negative strains were oral isolates and genotype 1, and they also yielded negative identifying characteristics in CH-P medium (Sahand et al., 2005),

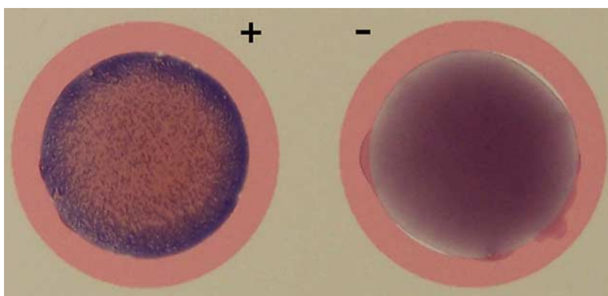

Fig. 1. Bichro-Dubli Fumouze® latex agglutination test showing positive (*C. dubliniensis*) and negative (*C. albicans*) results.

Table 1
Bichro-Dubli Fumouze® test results for the *Candida* strains tested

Species	No. of strains tested	Bichro-Dubli Fumouze® positive results
C. dubliniensis	106	103
C. albicans	30	0
C. krusei	1	0
C. tropicalis	1	0
C. glabrata	1	0
C. parapsilosis	1	0
C. guilliermondii	1	0
C. lusitaniae	1	0
C. kefyr	1	0
Total	143	103

although their identity as *C. dubliniensis* was reconfirmed by DNA sequencing after PCR with NL-1 and NL-4 primers that amplify the divergent domain at the 5′ end of the large-subunit (26S) ribosomal DNA gene (Kurtzman and Robnett, 1997). All the 30 *C. albicans* isolates assayed gave a negative result, as well as the other *Candida* species included in this study. Results were reproducible and independent of the medium in which colonies had been grown previously.

In our experience, the sensitivity of the test is 97.1% and the specificity is 100%. These data are in agreement with those provided by the Fumouze Diagnostics web site (http://www.biotribune.com/innovation/index.php?Mode=Detail&idInnov=53), where sensitivity is 97–100% and specificity is 100%.

In conclusion, the Bichro-Dubli Fumouze® test is a very rapid, easy to perform, simple and accurate assay for the identification of *C. dubliniensis*, regardless of the medium used for its isolation.

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