Comparative evaluation of ATB Fungus 2 and Sensititre YeastOne panels for testing in vitro *Candida* antifungal susceptibility

Elena Eraso[1], Maite Ruesga[1], María Villar-Vidal[1], Alfonso Javier Carrillo-Muñoz[2], Ana Espinel-Ingroff[3] and Guillermo Quindós[1]

[1]Laboratorio de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Apartado 699, E-48080 Bilbao, Spain; [2]Departamento de Microbiología, ACIA, P.O. Box 10178, E-08010 Barcelona, Spain; [3]Virginia Commonwealth University Medical Center, 1101 E. Marshall Street, Sanger Hall, Room 7-049, P.O. Box 980049, Richmond, VA 23298-0049, USA

Summary ATB Fungus 2 and SensititreYeastOne are commercial methods for antifungal susceptibility testing of yeasts. The agreement between these two methods was assessed with a total of 133 *Candida* strains (60 *Candida albicans*, 18 *Candida dubliniensis*, 29 *Candida glabrata*, and 26 *Candida krusei*). MIC endpoints were established after 24 h of incubation at 36 ± 1 ºC by each method. Intra-laboratory reproducibility of both methods was excellent (⩾ 99%). Overall agreement between ATB Fungus 2 and Sensititre YeastOne 3 MICs (within 2 dilutions) was 91.2-97.7% for amphotericin B, 5-fluorocytosine and itraconazole, and 82.7% for fluconazole. The categorical agreement when ATB Fungus 2 results were compared to those by SensititreYeastOne 3 was 93.2-98.5% for 5-fluorocytosine and amphotericin B, but lower for the triazoles (72.9-75.9%). This easy to perform method could be an alternative for routine use in the clinical microbiology laboratory for susceptibility testing of common *Candida* spp.

Key words ATB Fungus 2, Sensititre YeastOne, In vitro antifungal susceptibility, *Candida*

Evaluación comparativa de ATB Fungus 2 y Sensititre YeastOne en el estudio de la sensibilidad in vitro de *Candida* a los antifúngicos

Resumen ATB Fungus 2 y SensititreYeastOne son métodos comerciales para el estudio de la sensibilidad in vitro de levaduras a los antifúngicos. La concordancia entre estos dos métodos fue evaluada con un total de 133 aislamientos de *Candida* (60 *Candida albicans*, 18 *Candida dubliniensis*, 29 *Candida glabrata* y 26 *Candida krusei*). La lectura de las CMIs se realizó para cada método después de 24 h de incubación a 36 ± 1 ºC. La reproducibilidad intralaboratorio de ambos métodos fue excelente (⩾ 99%). La concordancia global entre las CMIs de ATB Fungus 2 y Sensititre YeastOne 3 (en un rango ± 2 diluciones) fue de 91,2-97,7% para anfotericina B, 5-fluorocitosina e itraconazol, y 82,7% para fluconazol. La concordancia por categorías cuando los resultados de ATB Fungus 2 fueron comparados con los de SensititreYeastOne 3 fue de 93,2-98,5% para 5-fluorocitosina y anfotericina B, pero más baja para los triazoles (72,9-75,9%). Este método sencillo de realizar puede ser una alternativa para uso de rutina en el laboratorio de microbiología clínica en el estudio de la sensibilidad de *Candida* spp.

Palabras clave ATB Fungus 2, Sensititre YeastOne, Sensibilidad in vitro a los antifúngicos, *Candida*

Corresponding author:
Dr. Guillermo Quindós
Laboratorio de Micología médica
Departamento de Inmunología, Microbiología y Parasitología
Facultad de Medicina y Odontología, Universidad del País Vasco
Apartado 699
E-48080 Bilbao, Spain
Tel.: +34 946012854
Fax: +34 946013495
E-mail: guillermo.quindos@ehu.es

1130-1406/01/10.00 €

Candida albicans is the most commonly species isolated from patients with candidiasis. Other species such as *Candida glabrata, Candida parapsilosis, Candida krusei,* or *Candida dubliniensis* are being frequently isolated from immunocompromised or surgically treated patients. A high variability in the susceptibility of clinical isolates to antifungal agents has been reported among these *Candida* spp. [7,8,10,16], emphasizing the importance of performing species identification and antifungal susceptibility testing [8,16].

In 2002, the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) and the European Committee on Antibiotic Susceptibility (EUCAST) proposed two broth dilution reference methods [2,4,9] for antifungal susceptibility testing of yeasts of clinical importance, but these standardized methods are time-consuming and cumbersome for routine use in the clinical laboratory. However, in recent years, a number of commercial methods have been introduced as easy-to-use, rapid alternatives [2,3,5,14,15]. One of these commercial method is the ATB Fungus 2, which allows the determination of the susceptibility of *Candida* spp. and *Cryptococcus neoformans* to antifungal agents in a semi-solid medium under conditions similar to those described in both EUCAST and CLSI reference methods [2,12,13,15].

The aim of this study was to evaluate the performance of the ATB Fungus 2 panel (bioMérieux, Marcy l'Étoile, France) in comparison to the Sensititre YeastOne 3 (Trek Diagnostic Systems, East Grinstead, UK) for the in vitro antifungal susceptibility testing of clinically important *Candida* isolates.

Materials and Methods

Yeast isolates. We tested eight reference strains (*C. albicans,* ATCC 76615, ATCC 90028, ATCC 90029, and NCPF 3153; *C. dubliniensis,* NCPF 3949, and CECT 11473; and *C. glabrata,* ATCC 90030, and NCPF 3240) and 125 clinical isolates including 56 *C. albicans,* 16 *C. dubliniensis,* 27 *C. glabrata,* and 26 *C. krusei;* susceptible (S), susceptible dose dependent (SDD), intermediate (I) and or resistant (R) isolates to the antifungal agents evaluated were included in the set. The isolates were identified by conventional mycological methods such as, the germ tube induction test in serum, microscopical morphology and chlamydospore formation in corn meal agar with Tween 80, and carbon source assimilation by the API ID 32C (bioMérieux) [1]. CLSI QC isolates *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as control isolates. Prior to testing, each strain was subcultured for 24-48 h at 36 ± 1 ºC on Sabouraud glucose agar (Difco, St. Louis, MO) and Candida ID2 (bioMérieux) to ensure viability and purity.

Susceptibility testing. The antifungal susceptibility of the study and QC isolates was evaluated by both ATB Fungus 2 (bioMérieux) and Sensititre YeastOne 3 (Trek Diagnostic Systems) methods. MIC endpoints of each isolate were determined after 24 h of incubation at 36 ± 1 ºC by both methods.

The ATB Fungus 2 panel (bioMérieux) consists of 16 pairs of wells. The first pair does not contain any antifungal agent and is used as growth control. The next 15 pairs contain four antifungal agents at the following drug concentrations: 5-fluorocytosine 0.5-64 µg/ml, amphotericin B 0.5-16 µg/ml, fluconazole 0.25-128 µg/ml, and itraconazole 0.125-4 µg/ml. The ATB Fungus 2 panel was evaluated by following the manufacturer's instructions [2,15]. Briefly, 20 µl of a 2 McFarland standard yeast suspension was added to the specific growth medium (ATB Fungus 2 medium). After the homogenization step, each well was inoculated with 135 µl of the homogenized inoculum. Following 24h of incubation at 36 ± 1 ºC, growth in the strips was read visually by two independent readers. According to the manufacturer's instructions, the amphotericin B MIC corresponded to the lowest concentration enabling complete growth inhibition. A certain amount of trailing growth was disregarded for fluconazole, itraconazole and 5-fluorocytosine and the MICs were the lowest drug concentrations that showed a prominent reduction in growth [2,15].

Sensititre YeastOne 3 (Trek Diagnostic Systems) contains several antifungal drugs, but only the four present in the ATB Fungus 2 strip were evaluated at the following drug concentrations: 5-fluorocytosine 0.03-64 µg/ml, amphotericin B 0.008-16 µg/ml, fluconazole 0.125-256 µg/ml, and itraconazole 0.008-16 µg/ml. The Sensititre YeastOne 3 antifungal panel was also evaluated according to the manufacturer's instructions. Briefly, 20 µl of a 0.5 McFarland standard yeast suspension was transferred into 11 ml of the RPMI broth tube and each well of the panel was inoculated with 100 µl of the diluted inoculum. The plates were visually read after 24 h of incubation at 36 ± 1 ºC. Growth in each well was indicated by a color change from blue (no growth) to red (growth). For amphotericin B, the MIC corresponded to the lowest concentration enabling complete growth inhibition (first blue well). For the other agents, the MIC was defined as the lowest drug concentration preventing the development of the red color (or first blue or purple well) corresponding to a significant inhibition of fungal growth.

Data analysis. Both on-scale and off-scale MICs by each method were included in the analysis. The evaluation of the reproducibility was based on the comparison of MICs of each antifungal agent obtained for each strain and by each method, as well as both types of reading by the ATB Fungus 2 panel. MICs were considered in agreement when the values were within no more than 2 dilutions. The performance of the ATB Fungus 2 panel in identifying resistant isolates was evaluated by determining the categorical agreement between the two methods by using CLSI M27-A2 MIC breakpoint categories [4-6,8-10]. A strain was considered -R- to 5-fluorocytosine, fluconazole, and itraconazole, if the MICs were $\geqslant$ 32, $\geqslant$ 64 and $\geqslant$ 1 µg/ml, respectively; -SDD- or -I- (only for 5-fluorocytosine) if the MICs were between 8-16, 16-32, and 0.25-0.5 µg/ml, respectively; and -S-, if the MICs were $\leqslant$ 4, $\leqslant$ 8 and $\leqslant$ 0.12 µg/ml, respectively. Breakpoints are not available for amphotericin B against any fungal species; however, based on serum concentrations, an isolate was considered -R- if the MIC was $\geqslant$ 1 µg/ml and -S- if the MIC was < 1 µg/ml. Discrepancies of MIC endpoints between the two methods were considered major errors when the isolate was R by the ATB Fungus 2 but -S- by the Sensititre YeastOne 3, while minor errors were identified when there were categorical shifts between -S- and -SDD- or between R and -SDD-. Very major errors were identified when the ATB Fungus 2 categorized an isolate as -S- and the Sensititre YeastOne 3 as -R-.

Results and Discussion

Different ready-to-use kits have been commercialized for antifungal susceptibility testing of clinically important yeasts [5,6,14]. The ATB Fungus 2 panel is an improved version of the original panel, because it includes fluconazole and itraconazole, two of the most widely used

antifungal drugs in therapy. Although both ATB Fungus 2 and Sensititre YeastOne 3 have demonstrated good to excellent agreements with the CLSI M27 method in different studies [2,5,13,15], limited data are available regarding the agreement of results obtained by these methods. Because of that we have evaluated the reproducibility of MICs results obtained by both methods.

In the present study, MIC readings for the two QC strains were within the reference limits described in the CLSI M27-A2 document [9] and in the guides for both commercial methods. Replicate testing of the QC strains also demonstrated excellent agreement between both methods with each antifungal agent (⩾ 99%). The reproducibility of MIC results for 8 of the 133 study strains that were tested in triplicate in each sequential test was excellent by each method (⩾ 99% global reproducibility). The agreement (no more than two dilutions) of MIC results obtained by ATB Fungus 2 and Sensititre YeastOne 3 for the 133 strains was good to excellent (91.7-97.7%) for three of the four agents (Table). The agreement was lower for fluconazole (82.7%).

The categorical agreement was good to excellent (93.2 to 98.5%) with 5-fluorocytosine and amphotericin B, but lower with both triazoles (72.9-75.9%) (Table). Although the majority of discrepant results were due to minor errors, the categorical evaluation indicated that the ATB Fungus 2 failed to identify 3 of the 12 itraconazole and 4 of the 21 fluconazole resistant isolates; no very major errors were observed with amphotericin B. Most minor errors were also due to higher fluconazole and itraconazole MIC results by Sensititre YeastOne 3 than by the ATB Fungus 2 (18 and 11 fluconazole and itraconazole SDD results, respectively, were S). Minor errors were below 7% with 5-fluorocytosine. In two recent reports [2,15], the comparison between CLSI or EUCAST reference methods with either Sensititre YeastOne 3 or ATB Fungus 2 have demonstrated excellent agreement (> 90%) between these methods for all antifungal agents tested, except for itraconazole (88%).

The present study mirrors these results for amphotericin B and 5-fluorocytosine, but in contrast the agreement was lower with fluconazole (82.7%) and higher with itraconazole (91.7%) (Table).

In summary, ATB Fungus 2 is a simple, effective and reproducible method and may be a valuable alternative for testing the antifungal activity of 5-fluorocytosine and amphotericin B against commonly isolated *Candida* spp.

Table. Essential agreement between ATB Fungus 2 and Sensititre YeastOne panels.

	Antifungal agents			
	5FC	AMB	FLC	ITC
MIC agreement				
Equivalent MIC	127/133 (95.5%)	130/133 (97.7%)	110/133 (82.7%)	122/133 (91.7%)
Different MICs	6/133 (4.5%)	3/133 (2.3%)	23/133 (17.3%)	11/133 (8.3%)
Category agreement				
Total agreement	124/133 (93.2%)	131/133 (98.5%)	97/133 (72.9%)	101/133 (75.9%)
Minor errors	9/133 (6.8%)	0/133 (NA)	30/133 (22.6%)	23/133 (17.3%)
Major errors	0/133 (NA)	2/133 (1.5%)	2/133 (1.5%)	6/133 (4.5%)
Very major errors	0/133 (0%)	0/133 (0%)	4/133 (3%)	3/133 (2.3%)

5FC= 5-fluorocytosine, AMB= amphotericin B, FLC= fluconazole, and ITC= itraconazole. NA=not applicable.

The results were less favorable for testing fluconazole and itraconazole due the false susceptible results for 3 of the 6 fluconazole -R- and 3 of the 12 itraconazole -R- isolates. Further comparisons with one of the two reference methods (CLSI or EUCAST) should clarify this issue since false-susceptible (very major errors) have been reported when the CLSI method has been compared to the Sensititre YeastOne when testing fluconazole [11].

The authors were financed in part with grants PI030662, and PI061895 from Fondo de Investigación Sanitaria and and IT-222-07 from Gobierno Vasco-Eusko Jaurlaritza. We are grateful to Ignacio Urrechaga and José Manuel Torres, from bioMérieux España, for the donation of the ATB Fungus 2 panels used in this study.

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