JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2007, p. 3522–3528 Vol. 45, No. 11
0095-1137/07/$08.00+0 doi:10.1128/JCM.00403-07

Multicenter Comparison of the VITEK 2 Antifungal Susceptibility Test with the CLSI Broth Microdilution Reference Method for Testing Amphotericin B, Flucytosine, and Voriconazole against *Candida* spp.▽

M. A. Pfaller,[1]* D. J. Diekema,[1] G. W. Procop,[2]† and M. G. Rinaldi[3]

University of Iowa College of Medicine, Iowa City, Iowa[1]; Cleveland Clinic Foundation, Cleveland, Ohio[2]; and University of Texas Health Science Center, San Antonio, Texas[3]

Received 21 February 2007/Returned for modification 17 April 2007/Accepted 18 June 2007

A fully automated commercial antifungal susceptibility test system (VITEK 2; bioMérieux, Inc., Hazelwood, MO) was compared in three different laboratories with the Clinical and Laboratory Standards Institute (formerly the NCCLS) reference broth microdilution method (BMD) by testing 2 quality control strains, 10 reproducibility strains, and 426 isolates of *Candida* spp. against amphotericin B, flucytosine, and voriconazole. Reference BMD MIC endpoints were established after 24 and 48 h of incubation. VITEK 2 system MIC endpoints were determined spectrophotometrically after 9.1 to 27.1 h of incubation (mean, 12 to 14 h). Excellent essential agreement (within 2 dilutions) between the VITEK 2 system and the 24- and 48-h BMD MICs was observed for all three antifungal agents: amphotericin B, 99.1% and 97%, respectively; flucytosine, 99.1% and 98.8%, respectively; and voriconazole, 96.7% and 96%, respectively. Both intra- and interlaboratory agreements were >98% for all three drugs. The overall categorical agreements between the VITEK 2 system and BMD for flucytosine and voriconazole were 98.1 to 98.6% at the 24-h BMD time point and 96.9 to 97.4% at the 48-h BMD time point. The VITEK 2 system reliably detected flucytosine and voriconazole resistance among *Candida* spp. and demonstrated excellent quantitative and qualitative agreement with the reference BMD method.

Standardized broth microdilution (BMD) susceptibility testing of *Candida* spp. against amphotericin B, flucytosine, fluconazole, and itraconazole has been available since 1997 (20, 25, 37) and against voriconazole since 2002 (21, 25, 36, 37). The establishment of a panel of quality control strains and validated, clinically useful, interpretive breakpoints (5, 27, 29, 35, 38) has allowed this method to be used worldwide (3, 6, 9, 10, 18, 41).

The Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) BMD method for the testing of amphotericin B, flucytosine, fluconazole, and voriconazole has served as the reference standard for the development of both broth- and agar-based procedures designed to provide simple, flexible, and commercially available alternative susceptibility testing methods for use in the clinical laboratory (7, 12, 13, 17, 18, 25, 26, 30, 31, 33). Although a number of antifungal testing systems are now commercially available, the performance has been variable (7, 12, 13, 17, 18, 26, 34). The Sensititre YeastOne system (Trek Diagnostic Systems, Cleveland, OH) was the first testing system approved by the U.S. Food and Drug Administration (FDA) for the in vitro susceptibility testing of *Candida* spp. against fluconazole, itraconazole, and flucytosine (25, 34). The YeastOne system is a colorimetric BMD panel that also contains amphotericin B and voriconazole (26). The Etest stable agar gradient method (AB Biodisk, Solna, Sweden) has also been approved by FDA for the in vitro susceptibility testing of *Candida* spp. against fluconazole and itraconazole (17).

With a view toward the future automation of antifungal susceptibility testing of fungi, considerable work has gone into examining the use of spectrophotometry as a potentially more rapid and objective means of determining MIC endpoints (2, 10, 17, 22, 40). The spectrophotometric approach to antifungal susceptibility testing has indeed been shown to be valid and feasible for use in the clinical laboratory (9–11) and is an integral component of the European Committee on Antibiotic Susceptibility Testing BMD method (8, 39). Until recently, spectrophotometry has not been incorporated into any commercially available testing method, leaving laboratories to contend with a nonautomated approach to antifungal susceptibility testing that is considered by many to be difficult and tedious to perform (25, 34). However, bioMerieux, Inc. (Hazelwood, MO), recently announced the development of an antifungal susceptibility test that determines growth spectrophotometrically and that allows fully automated antifungal susceptibility testing of *Candida* spp. by use of the VITEK 2 microbiology system (42). The fully automated VITEK 2 system allows the standardization of all the critical parameters known for antifungal susceptibility testing: inoculum preparation, filling of the device, the duration and temperature of incubation, and MIC endpoint determination. The antifungal susceptibility test, coupled with the rapid and accurate fungus identification

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, 200 Hawkins, Dr., Iowa City, IA 52242. Phone: (319) 384-9566. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.
† Present address: University of Miami, Miami, FL.
▽ Published ahead of print on 3 October 2007.

capabilities already available on the VITEK 2 system (4), would allow clinical laboratories to perform both fungal identification and antifungal susceptibility testing simultaneously using a fully automated and completely standardized format. Preliminary studies by Zambardi et al. (42) have shown both essential agreement (EA) and categorical agreement of ≥90% in a comparison of VITEK 2 system MICs with reference BMD MICs for amphotericin B, flucytosine, fluconazole, and voriconazole. In a recent multicenter evaluation of the VITEK 2 system for fluconazole, we demonstrated a high level of reproducibility (100%) and EAs of 93.7 to 97.9% compared to the reference BMD results when we tested 426 isolates of *Candida* spp. (28). Notably, the VITEK 2 system fluconazole MIC results were available within 10 to 26 h of incubation (mean, 13 h). In view of this excellent performance, the FDA approved the clinical use of the VITEK 2 system with fluconazole in the United States (bioMérieux press release, 27 September 2006).

The purpose of the present study was to further expand the number of antifungal agents available for testing on the VITEK 2 system. The performance of the VITEK 2 system with amphotericin B, flucytosine, and voriconazole was evaluated against a broad range of *Candida* spp. in three independent laboratories. The results obtained with the VITEK 2 system were compared with those obtained with a frozen reference BMD panel performed according to CLSI guidelines.

MATERIALS AND METHODS

Study design. We compared the MIC results for amphotericin B, flucytosine, and voriconazole obtained with the VITEK 2 system to those obtained by the CLSI M27-A2 BMD method (21) in three laboratories. Each laboratory tested at least 100 clinical isolates of *Candida* spp. (range, 103 to 135 isolates) with the VITEK 2 system and the CLSI frozen reference BMD panel (a total of 346 clinical isolates). In addition, a challenge set of 80 well-characterized stock isolates was tested by both methods in one of the laboratories. The intra- and interlaboratory reproducibilities were determined by testing a panel of 10 *Candida* spp. isolates in triplicate on three separate days in each of the participating laboratories. The MIC results obtained with the VITEK 2 system following 9.1 to 27.1 h of incubation (depending on the organism growth rate) were compared with those obtained with the reference BMD panel read after both 24 and 48 h of incubation.

Test organisms. The test organisms included two American Type Culture Collection (ATCC) strains that have been established as quality control strains (*Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) by the CLSI (5, 21). A challenge set of 80 isolates of *Candida* spp. selected to provide on-scale MIC results and to represent both clinically important species and resistance mechanisms were tested in one of the laboratories. The challenge set included 32 isolates of *C. albicans*, 6 of *C. dubliniensis*, 14 of *C. glabrata*, 3 of *C. guilliermondii*, 5 of *C. krusei*, 5 of *C. lusitaniae*, 1 of *C. norvegensis*, 7 of *C. parapsilosis*, 2 of *C. pelliculosa*, and 5 of *C. tropicalis*. An additional 346 recent clinical isolates of *Candida* spp. were also tested. The clinical isolates included 166 isolates of *C. albicans*, 2 of *C. dubliniensis*, 69 of *C. glabrata*, 46 of *C. krusei*, 4 of *C. lusitaniae*, 36 of *C. parapsilosis*, and 23 of *C. tropicalis*. Reproducibility within and among laboratories was assessed by using a panel of 10 *Candida* isolates: *C. glabrata* strain 304201, *C. glabrata* strain 304927, *C. haemulonii* strain 304848, *C. krusei* strain 304204, *C. krusei* strain 304845, *C. krusei* strain 304850, *C. lipolytica* strain 204856, *C. lusitaniae* strain 304205, *C. norvegensis* strain 304852, and *C. pelliculosa* strain 304847. All isolates were identified by standard methods (15). Before the tests were performed, each isolate was passaged at least twice on Sabouraud dextrose agar (Remel, Lenexa, KS) to ensure its purity and viability.

Antifungal agents and microdilution panels. The VITEK 2 cards containing serial twofold dilutions of amphotericin B (concentration range, 0.25 to 16 μg/ml), flucytosine (concentration range, 1 to 64 μg/ml), and voriconazole (concentration range, 0.125 to 8 μg/ml) were provided by the manufacturer. The frozen BMD reference panels containing serial twofold dilutions of amphotericin B (concentration range, 0.03 to 16 μg/ml), flucytosine (concentration range, 0.125 to 64 μg/ml), and voriconazole (concentration range, 0.03 to 16 μg/ml) were provided by Trek Diagnostic Systems. The VITEK 2 cards were shipped in sealed packages and were stored at 2 to 8°C until testing was performed. The BMD panels were shipped frozen in sealed packages and were stored at −70°C until the day of the test.

Inoculum preparation. Stock inoculum suspensions of the *Candida* spp. were obtained from 24-h cultures on Sabouraud dextrose agar at 35°C. Inoculum suspensions for the VITEK 2 system were prepared in sterile saline to a turbidity equal to a 2.0 McFarland standard by using the bioMérieux DensiChek instrument. The inoculum suspensions for the reference BMD were prepared by diluting a portion of the 2.0 McFarland suspension prepared for the VITEK 2 system to match the turbidity of a 0.5 McFarland standard.

CLSI BMD. Reference BMD testing was performed exactly as outlined in CLSI document M27-A2 (21). The panels were incubated in air at 35°C and were observed for the presence or the absence of growth at 24 and 48 h. The amphotericin B MIC was read as the lowest concentration that produced the complete inhibition of growth, and the flucytosine and voriconazole MICs were read as the lowest concentrations that produced a prominent decrease in turbidity (an approximately 50% reduction in growth) relative to the growth for the drug-free control (21).

VITEK 2 system. The standardized 2.0 McFarland inoculum suspension was placed into a VITEK 2 cassette along with a sterile polystyrene test tube and an antifungal susceptibility test card for each organism. The loaded cassettes were then placed into the VITEK 2 instrument; and the respective yeast suspensions were diluted appropriately by the instrument, after which the cards were filled, incubated, and read automatically. The time of incubation varied from 9.1 to 27.1 h, based on the rate of growth in the drug-free control well, and the results were expressed as MICs in μg/ml.

Quality control. Quality control was ensured by testing the CLSI-recommended quality control strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 (5, 21). These isolates were tested between 23 and 30 times in each of the three laboratories (total number of results = 324), with ≥95% of the MICs being within the respective reference ranges, as required.

Analysis of results. The MIC results obtained with the VITEK 2 system were compared with those of the reference BMD panels read at 24 h and 48 h. As with previous studies (10–12, 26), high off-scale MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies among MIC endpoints of more than 2 dilutions (two wells) were used to calculate the EA. Interlaboratory and intralaboratory agreements, assessed with the 10-isolate reproducibility panel, were defined when the MIC results were within a 3-dilution range (mode ± 1 $\log_2$ dilution). The CLSI interpretive breakpoints for flucytosine (susceptible, ≤ 4 μg/ml; intermediate, 8 to 16 μg/ml; resistant, ≥32 μg/ml) and for voriconazole (susceptible, ≤1 μg/ml; susceptible dose dependent, 2 μg/ml; resistant, ≥4 μg/ml) were used to obtain categorical agreement percentages between the MICs determined with the VITEK 2 system and by the reference BMD (21, 29, 37). Interpretive breakpoints for amphotericin B have not been defined by CLSI (21, 24); thus, only EA was determined for this agent. Very major errors were identified when the reference MIC indicated resistance and the VITEK 2 system MIC was susceptible. Major errors were identified when the isolate was classified resistant by the VITEK 2 system and susceptible by the reference method. Minor errors were determined when the result of one of the test methods was either susceptible or resistant and that of the other was intermediate (flucytosine) or susceptible dose dependent (voriconazole).

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 426 isolates of *Candida* spp. to amphotericin B, flucytosine, and voriconazole, as determined with the VITEK 2 system and by the reference BMD read at 24 and 48 h. Due to the similarity in the results obtained with the VITEK 2 system compared with those obtained with the 24 and 48 h BMD for both the challenge isolates (80 isolates; 93.8 to 100% EA) and the clinical isolates (346 isolates; 96.5 to 99.1% EA) the results for the two organism sets were combined in Table 1. In general, the MIC results for all three agents were typical of those for each species of *Candida* (25, 29, 32, 35). The BMD MICs read at 24 h of incubation tended to be approximately twofold lower than those read at 48 h for each of the three antifungal agents.

TABLE 1. In vitro susceptibilities of 426 isolates of *Candida* spp. to amphotericin B, flucytosine, and voriconazole as determined with the VITEK 2 system and by the CLSI BMD[a]

Species (no. of isolates tested)	Antifungal agent	Test method[b]	MIC (μg/ml)			EA (%)[c]
			Range	50%	90%	
C. albicans (198)	Amphotericin B	VITEK 2	≤0.25–≥16	0.5	0.5	
	Amphotericin B	BMD-24	0.125–2	1	1	100
	Amphotericin B	BMD-48	0.5–4	1	2	98.0
	Flucytosine	VITEK 2	≤1–≥64	≤1	≤1	
	Flucytosine	BMD-24	≤0.125–≥128	≤0.125	0.05	98.0
	Flucytosine	BMD-48	≤0.125–≥128	0.25	4	98.0
	Voriconazole	VITEK 2	≤0.125–≥8	≤0.125	≤0.125	
	Voriconazole	BMD-24	≤0.03–≥32	≤0.03	≤0.03	98.0
	Voriconazole	BMD-48	≤0.03–≥32	≤0.03	≤0.03	97.5
C. glabrata (83)	Amphotericin B	VITEK 2	≤0.25–4	0.5	1	
	Amphotericin B	BMD-24	0.25–4	2	2	100
	Amphotericin B	BMD-48	0.5–4	2	2	100
	Flucytosine	VITEK 2	≤1–4	≤1	≤1	
	Flucytosine	BMD-24	≤0.125–2	≤0.125	≤0.125	100
	Flucytosine	BMD-48	≤0.125–4	≤0.125	0.25	100
	Voriconazole	VITEK 2	≤0.125–≥8	0.25	1	
	Voriconazole	BMD-24	≤0.03–8	0.125	1	88.0
	Voriconazole	BMD-48	≤0.03–8	0.5	2	96.4
C. krusei (51)	Amphotericin B	VITEK 2	0.5–2	0.5	1	
	Amphotericin B	BMD-24	1–2	2	2	100
	Amphotericin B	BMD-48	2–4	2	2	94.1
	Flucytosine	VITEK 2	≤1–16	8	16	
	Flucytosine	BMD-24	≤0.125–16	8	16	100
	Flucytosine	BMD-48	0.25–32	16	16	100
	Voriconazole	VITEK 2	≤0.125–0.5	≤0.125	≤0.125	
	Voriconazole	BMD-24	≤0.03–0.5	0.125	0.25	100
	Voriconazole	BMD-48	≤0.03–1	0.5	1	82.4
C. parapsilosis (43)	Amphotericin B	VITEK 2	≤0.25–1	0.5	0.5	
	Amphotericin B	BMD-24	0.25–1	1	1	100
	Amphotericin B	BMD-48	1–2	2	2	90.7
	Flucytosine	VITEK 2	≤1	≤1	≤1	
	Flucytosine	BMD-24	≤0.125–0.25	≤0.125	≤0.125	100
	Flucytosine	BMD-48	≤0.125–0.5	≤0.125	0.25	100
	Voriconazole	VITEK 2	≤0.125	≤0.125	≤0.125	
	Voriconazole	BMD-24	≤0.03–0.06	≤0.03	≤0.03	100
	Voriconazole	BMD-48	≤0.03–0.125	≤0.03	0.06	100
C. tropicalis (28)	Amphotericin B	VITEK 2	≤0.25–0.5	0.5	0.5	
	Amphotericin B	BMD-24	0.5–4	1	1	92.9
	Amphotericin B	BMD-48	1–4	2	2	89.3
	Flucytosine	VITEK 2	≤1–16	≤1	≤1	
	Flucytosine	BMD-24	≤0.125–16	≤0.125	≤0.125	100
	Flucytosine	BMD-48	≤0.125–16	0.25	0.5	100
	Voriconazole	VITEK 2	≤0.125–0.25	≤0.125	≤0.125	
	Voriconazole	BMD-24	≤0.03–05	≤0.03	0.125	100
	Voriconazole	BMD-48	≤0.03–1	0.06	0.125	100
C. lusitaniae (9)	Amphotericin B	VITEK 2	0.5–8	0.5		
	Amphotericin B	BMD-24	0.06–2	0.5		88.9
	Amphotericin B	BMD-48	0.25–2	1		100
	Flucytosine	VITEK 2	≤1–≥64	≤1		
	Flucytosine	BMD-24	≤0.125–≥128	≤0.125		100
	Flucytosine	BMD-48	≤0.125–≥128	≤0.125		88.9
	Voriconazole	VITEK 2	≤0.125	≤0.125		
	Voriconazole	BMD-24	≤0.03	≤0.03		100
	Voriconazole	BMD-48	≤0.03	≤0.03		100
C. dubliniensis (8)	Amphotericin B	VITEK 2	≤0.25–0.5	0.5		
	Amphotericin B	BMD-24	0.125–05	0.25		100
	Amphotericin B	BMD-48	0.5–1	1		100
	Flucytosine	VITEK 2	≤1	≤1		
	Flucytosine	BMD-24	≤0.125	≤0.125		100

Continued on facing page

TABLE 1—*Continued*

Species (no. of isolates tested)	Antifungal agent	Test method[b]	MIC (μg/ml)			EA (%)[c]
			Range	50%	90%	
	Flucytosine	BMD-48	≤0.125–0.5	≤0.125		100
	Voriconazole	VITEK 2	≤0.125	≤0.125		
	Voriconazole	BMD-24	≤0.03–0.06	≤0.03		100
	Voriconazole	BMD-48	≤0.003–0.125	≤0.03		100
C. guilliermondii (3)	Amphotericin B	VITEK 2	≤0.25–0.5	0.5		
	Amphotericin B	BMD-24	0.25–1	0.5		100
	Amphotericin B	BMD-48	1–2	1		100
	Flucytosine	VITEK 2	≤1	≤1		
	Flucytosine	BMD-24	≤0.125	≤0.125		100
	Flucytosine	BMD-48	≤0.125	≤0.125		100
	Voriconazole	VITEK 2	≤0.125	≤0.125		
	Voriconazole	BMD-24	≤0.03–0.125	0.125		100
	Voriconazole	BMD-48	0.125–0.25	0.25		100
C. pelliculosa (2)	Amphotericin B	VITEK 2	0.5	0.5		
	Amphotericin B	BMD-24	0.5–1	0.5		100
	Amphotericin B	BMD-48	1–2	1		100
	Flucytosine	VITEK 2	1	≤1		
	Flucytosine	BMD-24	≤0.125	≤0.125		100
	Flucytosine	BMD-48	≤0.125–0.25	≤0.125		100
	Voriconazole	VITEK 2	≤0.125	≤0.125		
	Voriconazole	BMD-24	≤0.03–0.06	≤0.03		100
	Voriconazole	BMD-48	0.06–0.125	0.06		100
C. norvegensis (1)	Amphotericin B	VITEK 2	0.5			
	Amphotericin B	BMD-24	0.25			100
	Amphotericin B	BMD-48	1			100
	Flucytosine	VITEK 2	≤1			
	Flucytosine	BMD-24	2			100
	Flucytosine	BMD-48	2			100
	Voriconazole	VITEK 2	≤0.125			
	Voriconazole	BMD-24	0.125			100
	Voriconazole	BMD-48	0.125			100
All *Candida* spp. (426)	Amphotericin B	VITEK 2	≤0.25–≥16	0.5	1	
	Amphotericin B	BMD-24	0.06–4	1	2	99.1
	Amphotericin B	BMD-48	0.25–4	2	2	97.0
	Flucytosine	VITEK 2	≤0.1–≥64	≤1	8	
	Flucytosine	BMD-24	≤0.125–≥128	≤0.125	8	99.1
	Flucytosine	BMD-48	≤0.125–≥128	0.25	8	98.8
	Voriconazole	VITEK 2	≤0.125–≥8	≤0.125	0.25	
	Voriconazole	BMD-24	≤0.03–≥32	≤0.03	0.25	96.7
	Voriconazole	BMD-48	≤0.03–≥32	≤0.03	0.5	96.0

[a] Isolates include both clinical (n = 346) and challenge (n = 80) sets.
[b] BMD-24 and BMD-48, BMD incubation for 24 and 48 h, respectively.
[c] EA (±2 $\log_2$ dilutions) between VITEK 2 and BMD MICs.

The overall EA between the VITEK 2 system and the BMD MICs ranged from 96.7% (voriconazole) to 99.1% (amphotericin B and flucytosine) when the 24-h BMD result was used as the reference and from 96.0% (voriconazole) to 98.8% (flucytosine) when the 48-h BMD result was used as the reference. Of the discrepancies noted between the VITEK 2 system and the 24-h BMD results, the MICs generated by the VITEK 2 system were higher than those obtained by BMD in 19 of 22 (86.4%) instances (2 of 4 with amphotericin B, 4 of 4 with flucytosine, 13 of 14 with voriconazole). In contrast, of the 35 discrepancies observed between the VITEK 2 system and 48-h BMD MIC results, the MICs generated by the VITEK 2 system were lower than those obtained by BMD in 31 instances (88.6%; 13 of 15 with amphotericin B, 5 of 5 with flucytosine, 13 of 17 with voriconazole). The largest number of discrepancies observed with the VITEK 2 system and 24-h BMD comparison occurred with *C. glabrata* tested against voriconazole (10 discrepant results), whereas the largest number of discrepancies seen with the VITEK 2 system and 48-h BMD comparison occurred with voriconazole and *C. krusei* (9 discrepant results).

The mean times to the results for the VITEK 2 system were 14.3 h with amphotericin B (range, 10.7 to 26.8 h), 14.0 h with flucytosine (range, 11.0 to 27.1 h), and 12.7 h with voriconazole (range, 9.1 to 26.3 h). Only one isolate, a clinical isolate of *C. parapsilosis*, failed to grow in the VITEK 2 system and all isolates grew sufficiently well in the BMD panel to be read after 24 h of incubation. Similar results were obtained at all three study sites.

Regarding the individual species of *Candida*, the EAs be-

TABLE 2. Categorical agreement between VITEK 2 system MICs and 24-h and 48-h BMD flucytosine and voriconazole MICs for 426 isolates of *Candida* spp. in three laboratories[a]

Species (no. of isolates tested)	Antifungal agent	Test method	% of MICs by category			CA (%)	% Errors		
			S	I/SDD	R		VME	ME	Minor
C. albicans (198)	Flucytosine	VITEK 2	97.0	0.0	3.0				
	Flucytosine	BMD-24	99.0	0.0	1.0	98.0	0.0	2.0	0.0
	Flucytosine	BMD-48	94.9	0.5	4.6	98.0	1.5	0.0	0.5
	Voriconazole	VITEK 2	98.0	0.5	1.5				
	Voriconazole	BMD-24	98.5	0.0	1.5	99.0	0.0	0.5	0.5
	Voriconazole	BMD-48	98.0	0.5	1.5	98.5	0.0	0.5	1.0
C. glabrata (83)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	90.4	0.0	9.6				
	Voriconazole	BMD-24	90.4	4.8	4.8	95.2	0.0	0.0	4.8
	Voriconazole	BMD-48	84.4	8.4	7.2	90.4	1.2	0.0	8.4
C. krusei (51)	Flucytosine	VITEK 2	21.6	78.4	0.0				
	Flucytosine	BMD-24	17.6	82.4	0.0	92.2	0.0	0.0	7.8
	Flucytosine	BMD-48	7.8	92.2	0.0	84.3	0.0	0.0	15.7
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. parapsilosis (43)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. tropicalis (28)	Flucytosine	VITEK 2	96.4	3.6	0.0				
	Flucytosine	BMD-24	96.4	3.6	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	96.4	3.6	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. lusitaniae (9)	Flucytosine	VITEK 2	88.9	0.0	11.1				
	Flucytosine	BMD-24	88.9	0.0	11.1	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	77.8	11.1	11.1	88.9	0.0	0.0	11.1
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. dubliniensis (8)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. guilliermondii (3)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. pelliculosa (2)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. norvegensis (1)	Flucytosine	VITEK 2	100.0	0.0	0.0				
	Flucytosine	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Flucytosine	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	VITEK 2	100.0	0.0	0.0				
	Voriconazole	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Voriconazole	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
All *Candida* spp. (426)	Flucytosine	VITEK 2	88.8	9.6	1.6				
	Flucytosine	BMD-24	89.2	10.1	0.7	98.2	0.0	0.9	0.9
	Flucytosine	BMD-48	85.9	11.5	2.6	97.0	0.7	0.0	2.3
	Voriconazole	VITEK 2	97.2	0.2	2.6				
	Voriconazole	BMD-24	97.4	1.0	1.6	98.6	0.0	0.2	1.2
	Voriconazole	BMD-48	96.0	1.9	2.1	97.5	0.2	0.2	2.1

[a] Abbreviations: S, susceptible; I, intermediate (for flucytosine only); SDD, susceptible dose dependent (for voriconazole only); R, resistant; CA, categorical agreement; VME, very major error; ME, major error; minor, minor error; BMD-24 and BMD-48, BMD method read at 24 h and 48 h of incubation, respectively. Isolates include both clinical (n = 346) and challenge (n = 80) sets.

tween the VITEK 2 system results and the 24-h BMD MICs were >90% for all organism-drug combinations, with the exception of *C. glabrata* and voriconazole (88.0% EA) and *C. lusitaniae* and amphotericin B (88.9% EA). Likewise, the EAs for the VITEK 2 system and 48-h BMD comparison were >90% for all organism-drug combinations, with the exception of *C. krusei* and voriconazole (82.4% EA), *C. tropicalis* and amphotericin B (89.3% EA), and *C. lusitaniae* and flucytosine (88.9% EA). The VITEK 2 system MIC results were highly predictive of the reference BMD results for all organism-drug combinations.

The VITEK 2 system MIC results for all three antifungal agents were highly reproducible, as determined by replicate testing of a panel of 10 *Candida* sp. isolates in the three laboratories (data not shown). Overall, 803 of 810 (99%) MIC results fell within a 3-dilution range (mode ± 1 dilution) for the three agents: voriconazole, 98.1% (265/270 results); flucytosine, 99.3% (268/270 results); and amphotericin B, 100% (270/270 results). This high level of reproducibility underscored the excellent level of test standardization achieved with this automated microbiology system.

The categorical agreement between the results obtained with the VITEK 2 system and those obtained by BMD with flucytosine and voriconazole was assessed by combining the data obtained with the clinical and challenge organism collections in all three laboratories (Table 2). Overall, 88.8% of the 426 isolates were susceptible to flucytosine (MICs ≤ 4 μg/ml), as determined with the VITEK 2 system, whereas 89.2% and 85.9% were susceptible by BMD read at 24 and 48 h, respectively. Likewise, the excellent activity of voriconazole (MICs ≤ 1 μg/ml) was seen with the VITEK 2 system (97.2% susceptible), the 24-h BMD (97.4% susceptible), and the 48-h BMD (96.0% susceptible). Although resistance was infrequent, comparable levels of resistance to flucytosine (0.7 to 2.6%) and voriconazole (1.6 to 2.6%) were detected by all three testing approaches.

Excellent categorical agreement was observed for all comparisons (Table 2). The overall categorical agreements for the comparison of the VITEK 2 system results with the 24-h BMD results were 98.2% for flucytosine and 98.6% for voriconazole, with no very major (false-susceptible) errors. None of the 10 species tested against either flucytosine or voriconazole showed less than 90% absolute categorical agreement with the 24-h BMD results. The only major (false-resistant) errors involving either antifungal agent occurred with *C. albicans*, whereas minor errors were seen with voriconazole and both *C. albicans* and *C. glabrata* and with flucytosine and *C. krusei.*

The overall categorical agreement between the VITEK 2 system and the 48-h BMD results for both flucytosine and voriconazole was only slightly lower than that seen with the VITEK 2 system and 24-h BMD comparison (97.0 to 97.5% versus 98.2 to 98.6%, respectively). The only very major errors were seen with *C. albicans* and flucytosine (3 of 198 results) and with *C. glabrata* and voriconazole (1 of 83 results). Minor error rates of >10% were observed only with *C. krusei* and flucytosine (15.7%) and with *C. lusitaniae* and flucytosine (11.1%). The latter two species were the only ones for which the categorical agreement fell below 90% for either agent tested.

The overall pattern of the results shown in Table 2 was also seen with clinical isolates tested in each of the three laboratories (data not shown). In each laboratory, the categorical agreement was >98% for both flucytosine (range, 98.1 to 100%) and voriconazole (range, 98.1 to 99.1%) when the VITEK 2 system results were compared with the 24-h BMD results and ranged from 94.2% to 100% with the VITEK 2 system and 48-h BMD comparison.

These findings demonstrate that, in addition to the recently FDA-approved susceptibility test for fluconazole (28), the VITEK 2 system also provides a means of determining the MICs for amphotericin B, flucytosine, and voriconazole when they are tested against *Candida* spp. This system is the first commercially available automated approach to antifungal susceptibility testing and provides optimal susceptibility test standardization. In addition to providing highly reproducible results that reliably predict the MICs determined by the reference BMD, the VITEK 2 system was rapid, with a mean time to results of 12 to 15 h. The availability of rapid, quantitative antifungal susceptibility data will be a major step in optimizing the therapy of invasive candidal infections (1, 14, 16, 19, 23).

In summary, the MICs of amphotericin B, flucytosine, voriconazole, and fluconazole can now be determined in an automated fashion in less than 15 h for most species of *Candida* with the VITEK 2 system. The VITEK 2 system ensures that each test is performed in a highly standardized fashion and provides quantitative MIC results that are reproducible and accurate. The use of spectrophotometry to determine the MIC endpoint eliminates the subjectivity that compromises the performance of systems that rely on visual MIC determination. The VITEK 2 system reliably identifies resistance to flucytosine and voriconazole, as well as to fluconazole (28), and demonstrates excellent quantitative and qualitative agreement with the reference BMD method.

ACKNOWLEDGMENTS

This study was supported in part by a grant from bioMérieux, Inc.

We thank Linda Elliot for secretarial assistance in the preparation of the manuscript. We acknowledge the excellent assistance of the technical personnel in the Iowa, Ohio, and Texas laboratories.

REFERENCES

1. **Alexander B. D., and M. A. Pfaller.** 2006. Contemporary tools for the diagnosis and management of invasive mycosis. Clin. Infect. Dis. **43**(Suppl. 1)**:** S15–S27.
2. **Arthington-Skaggs, B. A., W. Lee-Yang, M. A. Ciblak, et al.** 2002. Comparison of visual and spectrophotometric methods of broth microdilution MIC endpoint determination and evaluation of sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazole against trailing and nontrailing *Candida* isolates. Antimicrob. Agents Chemother. **46:**2477–2481.
3. **Asmundsdottir, L. R., H. Erlendsdottir, and M. Gotfredsson.** 2002. Increasing incidence of candidemia: results from a 20-year nationwide study in Iceland. J. Clin. Microbiol. **40:**3489–3492.
4. **Aubertine, C. L., M. Rivera, S. M. Rohan, and D. H. Larone.** 2006. Comparative study of the new colorimetric VITEK 2 yeast identification card versus the older fluorometric card and of CHROMagar Candida as a source medium with the new card. J. Clin. Microbiol. **44:**227–228.
5. **Barry, A. L., M. A. Pfaller, S. D. Brown, et al.** 2000. Quality control limits for broth microdilution susceptibility tests of ten antifungal agents. J. Clin. Microbiol. **28:**3457–3459.
6. **Chen, Y. C., S. C. Chang, K. T. Luh, and W. C. Hsieh.** 2002. Stable susceptibility of *Candida* bloodstream isolates to fluconazole despite increasing use during the past 10 years. J. Antimicrob. Chemother. **52:**71–77.
7. **Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado, and J. L. Rodriguez-Tudela.** 2005. Correlation between the procedure for antifungal susceptibility testing for *Candida* spp. of the European Committee on Antibiotic Sus-

ceptibility Testing (EUCAST) and four commercial techniques. Clin. Microbiol. Infect. **11:**486–492.
8. **Cuenca-Estrella, M., C. B. Moore, F. Barchiesi, et al.** 2003. Multicenter evaluation of the reproducibility of the proposed antifungal susceptibility method for fermentative yeasts of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST). Clin. Microbiol. Infect. **9:**467–474.
9. **Cuenca-Estrella, M., W. Lee-Yang, M. A. Ciblak, et al.** 2002. Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *Candida* species. Antimicrob. Agents Chemother. **46:**3644–3647.
10. **Espinel-Ingroff, A., F. Barchiesi, M. Cuenca-Estrella, et al.** 2005. Comparison of visual 24-hour and spectrophotometric 48-hour MICs to CLSI reference microdilution MICs of fluconazole, itraconazole, posaconazole, and voriconazole for *Candida* spp.: a collaborative study. J. Clin. Microbiol. **43:**4535–4540.
11. **Espinel-Ingroff, A., F. Barchiesi, M. Cuenca-Estrella, et al.** 2005. International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibility of *Candida* spp. to fluconazole, itraconazole, posaconazole, and voriconazole. J. Clin. Microbiol. **43:** 3884–3889.
12. **Espinel-Ingroff, A., M. A. Pfaller, S. A. Messer, et al.** 2004. Multicenter comparison of the Sensititre YeastOne colorimetric antifungal panel with the NCCLS M27-A2 reference method for testing new antifungal agents against clinical isolates of *Candida* spp. J. Clin. Microbiol. **42:**718–721.
13. **Espinel-Ingroff, A., M. A. Pfaller, S. A. Messer, et al.** 1999. Multicenter comparison of the Sensititre YeastOne colorimetric antifungal panel with the National Committee for Clinical Laboratory Standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeast and yeast-like organisms. J. Clin. Microbiol. **37:**591–595.
14. **Garey, K. W., M. Rege, M. P. Pai, et al.** 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. **43:**25–31.
15. **Hazen, K. C., and S. A. Howell.** 2003. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 1693–1711. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. ASM Press, Washington, DC.
16. **Magill, S. S., C. Shields, C. L. Sears, M. Choti, and W. G. Merz.** 2006. Triazole-cross resistance among *Candida* spp.: case report, occurrence among blood stream isolates, and implication for antifungal therapy. J. Clin. Microbiol. **44:**529–535.
17. **Matar, M. J., L. Ostrosky-Zeichner, V. L. Paetznick, et al.** 2003. Correlation between E-test disk diffusion, and microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole. Antimicrob. Agents Chemother. **47:**1647–1651.
18. **Morace, G., G. Amato, F. Bistoni, et al.** 2002. Multicenter comparative evaluation of six commercial systems and the National Committee for Clinical Laboratory Standards M27-A broth microdilution method for fluconazole susceptibility testing of *Candida* species. J. Clin. Microbiol. **40:**2953–2958.
19. **Morrell, M., V. J. Fraser, and M. J. Kollef.** 2005. Delaying empirical treatment of *Candida* bloodstream infection until positive blood culture results are obtained: a potential risk factor for mortality. Antimicrob. Agents Chemother. **49:**3640–3645.
20. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeast. M27-A. National Committee for Clinical Laboratory Standards, Villanova, PA.
21. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 2nd ed., M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.
22. **Nguyen M. H., and C. Y. Yu.** 1999. Influence of incubation time, inoculum size, and glucose concentrations on spectrophotometric endpoint determinations for amphotericin B, fluconazole and itraconazole. J. Clin. Microbiol. **37:**141–145.
23. **Pappas, P. G., J. H. Rex, J. D. Sobel, et al.** 2004. Guidelines for treatment of candidiasis. Clin. Infect. Dis. **38:**161–189.
24. **Park, B. J., B. A. Arthington-Skaggs, R. A. Hajjeh, et al.** 2006. Evaluation of amphotericin B interpretive breakpoints for *Candida* bloodstream isolates by correlation with therapeutic outcome. Antimicrob. Agents Chemother. **50:** 1287–1292.
25. **Pfaller, M. A.** 2005. Antifungal susceptibility testing methods. Curr. Drug Targets **6:**929–943.
26. **Pfaller, M. A., A. Espinel-Ingroff, and R. N. Jones.** 2004. Clinical evaluation of the Sensititre YeastOne colorimetric antifungal plate for antifungal susceptibility testing of the new triazoles voriconazole, posaconazole, and ravuconazole. J. Clin. Microbiol. **42:**4577–4580.
27. **Pfaller, M. A., D. J. Diekema, and D. J. Sheehan.** 2006. Interpretive breakpoints for fluconazole and *Candida* revisited: a blueprint for the future of antifungal susceptibility testing. Clin. Microbiol. Rev. **19:**435–447.
28. **Pfaller, M. A., D. J. Diekema, G. W. Procop, and M. G. Rinaldi.** 2007. Multicenter comparison of the VITEK 2 yeast susceptibility test with the CLSI broth microdilution reference method for testing fluconazole against *Candida* spp. J. Clin. Microbiol. **45:**796–802.
29. **Pfaller, M. A., D. J. Diekema, J. H. Rex, et al.** 2006. Correlation of MIC with outcome for *Candida* species against voriconazole: analysis and proposal for interpretive breakpoints. J. Clin. Microbiol. **44:**819–826.
30. **Pfaller, M. A., K. C. Hazen, S. A. Messer, et al.** 2004. Comparison of results of fluconazole disk diffusion testing for *Candida* species with results from a central reference laboratory in the ARTEMIS Global Antifungal Surveillance Program. J. Clin. Microbiol. **42:**3607–3612.
31. **Pfaller, M. A., L. Boyken, R. J. Hollis, et al.** 2004. Clinical evaluation of a dried commercially prepared microdilution panel for antifungal susceptibility testing of five antifungal agents against *Candida* spp. and *Cryptococcus neoformans*. Diagn. Microbiol. Infect. Dis. **50:**113–117.
32. **Pfaller, M. A., L. Boyken, S. A. Messer, et al.** 2004. Evaluation of the Etest method using Mueller-Hinton agar with glucose and methylene blue for determining amphotericin B MICs for 4,936 clinical isolates of *Candida* species. J. Clin. Microbiol. **42:**4977–4979.
33. **Pfaller, M. A., L. Boyken, S. A. Messer, et al.** 2005. Comparison of results of voriconazole disk diffusion testing for *Candida* species with results from a central reference laboratory in the ARTEMIS Global Antifungal Surveillance Program. J. Clin. Microbiol. **43:**5208–5213.
34. **Pfaller, M. A., and R. N. Jones, for the Microbiology Resource Committee of the College of American Pathologists.** 2006. Performance accuracy of antibacterial and antifungal susceptibility test methods: report from the College of American Pathologists (CAP) Microbiology Surveys Program (2001–2003). Arch. Pathol. Lab. Med. **130:**767–778.
35. **Pfaller, M. A., S. A. Messer, L. Boyken, et al.** 2002. In vitro activities of 5-fluorocytosine against 8,803 clinical isolates of *Candida* spp.: global assessment of primary resistance using National Committee for Clinical Laboratory Standards susceptibility testing methods. Antimicrob. Agents Chemother. **46:**3518–3521.
36. **Pfaller, M. A., S. A. Messer, R. J. Hollis, R. N. Jones, and D. J. Diekema.** 2002. In vitro activities of ravuconazole and voriconazole compared with those of four approved systematic antifungal agents against 6,970 clinical isolates of *Candida* spp. Antimicrob. Agents Chemother. **46:**1723–1727.
37. **Rex, J. H., M. A. Pfaller, J. N. Galgiani, et al.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. Clin. Infect. Dis. **24:**235–247.
38. **Rex, J. H., M. A. Pfaller, T. J. Walsh, et al.** 2001. Antifungal susceptibility testing: practical aspects and current challenges. Clin. Microbiol. Rev. **14:**643–658.
39. **Rodriguez-Tudela, J. L., F. Barchiesi, J. Bille, et al.** 2003. Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Clin. Microbiol. Infect. **9:**I–VIII.
40. **Rodriguez-Tudela, J. L., M. Cuenca-Estrella, T. M. Diaz-Guerra, and E. Mellado.** 2001. Standardization of antifungal susceptibility variables for a semiautomated methodology. J. Clin. Microbiol. **39:**2513–2517.
41. **Tortorano, A. M., A. L. Rigoni, E. Biraghi, et al.** 2003. The European Confederation of Medical Mycology (ECMM) survey of candidemia in Italy: antifungal susceptibility patterns of 261 non-*albicans Candida* isolates from blood. J. Antimicrob. Chemother. **52:**679–682.
42. **Zambardi, G., D. Parreno, V. Monnin, et al.** 2005. Rapid antifungal susceptibility testing of medically important yeasts with the VITEK 2 system, abstr. M-1619. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.