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Multicenter Comparison of the VITEK 2 Yeast Susceptibility Test with the CLSI Broth Microdilution Reference Method for Testing Fluconazole against *Candida* spp.$^{\nabla}$

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A fully automated commercial antifungal susceptibility test system (VITEK 2 yeast susceptibility test; bioMerieux, Inc., Hazelwood, Mo.) was compared in three different laboratories with Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method by testing two quality control strains and a total of 426 isolates of *Candida* spp. (103 to 135 clinical isolates in each laboratory plus 80 challenge isolates in one laboratory) against fluconazole. Reference BMD MIC endpoints were established after 24 and 48 h of incubation. VITEK 2 endpoints were determined spectrophotometrically after 10 to 26 h of incubation (mean, 13 h). Excellent essential agreement (within two dilutions) between the VITEK 2 and the 24- and 48-h BMD MICs was observed. The overall agreement values were 97.9 and 93.7%, respectively. Both intra- and interlaboratory agreement was 100%. The overall categorical agreement between VITEK 2 and BMD was 97.2% at the 24-h BMD time point and 88.3% at the 48-h BMD time point. Decreased categorical agreement at 48 h was attributed to trailing growth observed with *Candida glabrata*. The VITEK 2 system reliably detected fluconazole resistance among *Candida* spp. and demonstrated excellent quantitative and qualitative agreement with the reference BMD method.

Standardized broth microdilution (BMD) susceptibility testing of fluconazole against *Candida* spp. has been available since 1997 (19, 21, 30). The establishment of a panel of quality control (QC) strains and validated, clinically useful interpretive breakpoints (19, 20, 27, 30, 32) has allowed this method to be used worldwide (3, 6–8, 12, 17, 21, 33).

The Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards [NCCLS]) BMD method for testing fluconazole has served as a touchstone 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 (8–12, 16, 17, 21, 23–25). The performance of the various commercially available antifungal testing systems has been variable (8, 10, 16, 17), and prior to the present study only two, the Sensititre YeastOne System (Trek, Cleveland, OH) and the Etest (AB BIODISK, Solna Sweden), have been approved by the U.S. Food and Drug Administration (FDA) for in vitro susceptibility testing of fluconazole against *Candida* spp. (21, 26).

Although spectrophotometric reading of BMD MIC endpoints has been shown to be valid and feasible for use in the clinical laboratory (11, 12, 16, 20, 21), this approach has not been incorporated into a commercially available testing method. Recently, bioMerieux (Hazelwood, MO) has developed a yeast susceptibility test that determines growth spectrophotometrically and allows fully automated antifungal susceptibility testing of fluconazole against *Candida* using the VITEK 2 microbiology system. The fully automated VITEK 2 system allows for the standardization of all of the critical parameters known for antifungal susceptibility testing: inoculum preparation, filling of the device, duration and temperature of incubation, and endpoint determination. The yeast susceptibility test, coupled with the rapid and accurate yeast identification capabilities already available on the VITEK 2 (4), would allow clinical laboratories to perform both yeast identification and antifungal susceptibility testing using a fully automated and completely standardized format. Preliminary studies by Zambardi et al. (G. Zambardi et al., Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1619, 2005) have shown both essential and categorical agreements of ≥90% in a comparison of VITEK 2 MICs with reference BMD MICs for fluconazole and *Candida* spp. The VITEK 2 results were available in ≤15 h compared to 48 h for the reference BMD method.

The purpose of the present study was to validate the performance of the VITEK 2 yeast susceptibility test with fluconazole against a broad range of *Candida* spp. in three independent laboratories. The VITEK 2 results were compared to those from a frozen reference BMD panel performed according to CLSI guidelines. It is notable that after the completion of the present study the VITEK 2 yeast susceptibility test for fluconazole was approved for clinical use by the U.S. FDA (bioMerieux press release, 27 September 2006).

MATERIALS AND METHODS

Study design. The study was designed to compare the MIC results for fluconazole obtained by the VITEK 2 yeast susceptibility test to those obtained by the M27-A2 BMD method (20) in the three laboratories. Each laboratory tested at least 100 clinical isolates of *Candida* spp. (range, 103 to 135 isolates) by the VITEK 2 system and the CLSI frozen reference BMD panel (a total of 346

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clinical isolates). In addition, a challenge set of 80 well-characterized stock isolates was tested by both methods in one of the laboratories. Intra- and interlaboratory reproducibility was 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 after 10 to 26 h of incubation (depending on the organism growth rate) were compared to 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 QC strains (*Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) by the CLSI (5, 20). A challenge set of 80 isolates of *Candida* spp. selected to provide strains with on-scale MIC results and to represent both clinically important species and resistance mechanisms were tested in one of the participating laboratories. The challenge set included 32 isolates of *C. albicans*, 6 isolates of *C. dubliniensis*, 14 isolates of *C. glabrata*, 3 isolates of *C. guilliermondii*, 5 isolates of *C. krusei*, 5 isolates of *C. lusitaniae*, 1 isolate of *C. norvegensis*, 7 isolates of *C. parapsilosis*, 2 isolates of *C. pelliculosa*, and 5 isolates of *C. tropicalis.* An additional 346 clinical isolates of *Candida* spp. were also tested. The clinical isolates included 166 isolates of *C. albicans*, 2 isolates of *C. dubliniensis*, 69 isolates of *C. glabrata*, 46 isolates of *C. krusei*, 4 isolates of *C. lusitaniae*, 36 isolates of *C. parapsilosis*, and 23 isolates of *C. tropicalis*. These were all recent clinical isolates and were selected to represent the clinically prevalent species, including fluconazole-resistant strains. 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. These isolates were selected to provide on-scale fluconazole MICs ranging from 2 to 32 μg/ml. All isolates were identified by standard methods (14). Prior to testing, each isolate was passaged at least twice on Sabouraud dextrose agar (Remel, Lenexa, KS) to ensure purity and viability.

Antifungal agents and microdilution panels. The VITEK 2 cards containing serial twofold dilutions of fluconazole (range, 1 to 64 μg/ml) were provided by the manufacturer. The frozen BMD reference panels containing serial twofold dilutions of fluconazole (range, 0.12 to 128 μg/ml) were provided by Trek Diagnostic Systems (Cleveland, OH). The VITEK 2 cards were shipped in sealed packages and 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. The inoculum suspensions for the VITEK 2 were prepared in sterile saline to a turbidity equal to a 2.0 McFarland standard by using the bioMerieux 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 to match the turbidity of a 0.5 McFarland.

CLSI broth microdilution method. Reference BMD testing was performed exactly as outlined in CLSI document M27-A2 (20) with a final inoculum concentration of $1.5 \times 10^3 \pm 1.0 \times 10^3$ cells/ml and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer. The panels were incubated in air at 35°C and observed for the presence or absence of growth at 24 and 48 h. The fluconazole MIC was read as the lowest concentration that produced a prominent decrease in turbidity (ca. 50% reduction in growth) relative to the drug-free control (20).

VITEK 2 yeast susceptibility test. The standardized 2.0 McFarland inoculum suspension was placed into a VITEK 2 cassette along with a sterile polystyrene test tube and a yeast 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, after which the cards were filled, incubated, and read automatically. The time of incubation varied from 10 to 26.1 h, based on the rate of growth in the drug-free control well, and the results were expressed as MICs in micrograms per milliliter.

Quality control. Quality control was ensured by testing the CLSI-recommended quality control strains *C. parapsilosis* ATCC 2209 and *C. krusei* 6258 (5, 20). These isolates were tested between 22 and 29 times in each of the three laboratories (total number of results = 294), and all (100%) MICs were in the respective reference ranges.

Analysis of results. The MIC results obtained with the VITEK 2 yeast susceptibility test were compared to those of the reference BMD panels read at 24 and 48 h. As with previous studies (9–12, 24, 25), 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 two dilutions (two wells) were used to calculate the essential agreement (EA). Interlaboratory and intralaboratory agreement, assessed with the 10-isolate reproducibility panel, was defined when MIC results were within a three-dilution range. The CLSI interpretive breakpoints for fluconazole (susceptible [S], ≤8 μg/ml; susceptible dose dependent [SDD], 16 to 32 μg/ml; resistant [R], ≥64 μg/ml) were used to obtain categorical agreement (CA) percentages between the MICs determined by VITEK 2 and the reference BMD (20, 27). Very major errors (VME) were identified when the reference MIC indicated R, and the VITEK 2 MIC indicated S. Major errors (ME) were identified when the isolate was classified as R by the VITEK 2 and S by the reference method. Minor errors were determined when the results of one of the test methods was either S or R and that of the other was SDD. The MIC results obtained for *C. krusei* were used as such (in micrograms per milliliter) for the purpose of assessing EA but were forced into the R category, as required by the CLSI (20), when determining CA.

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro fluconazole susceptibilities of 426 isolates of *Candida* spp. as determined by the VITEK 2 system and the reference BMD read at 24 and 48 h. Due to the similarity in results obtained with the VITEK 2 compared to the 24- and 48-h BMDs for both the challenge isolates (80 isolates, 100 and 97.5% EA, respectively) and the clinical isolates (346 isolates, 97.4 and 92.8% EA, respectively), the results for the two organism sets were combined in Table 1. In general, MIC results for fluconazole were typical of each species of *Candida* (22), with the lowest MICs obtained with both VITEK 2 and BMD observed for *C. albicans* and the highest MICs observed for *C. glabrata* and *C. krusei*. Notably, BMD MICs read at 24 h of incubation tended to be approximately fourfold lower than those read at 48-h for both *C. glabrata* (MIC_{50}, 4 μg/ml versus 16 μg/ml, respectively) and *C. krusei* (MIC_{50}, 16 μg/ml versus 64 μg/ml, respectively), suggesting significant trailing with these two species.

The overall EA between the VITEK 2 and the BMD MICs ranged from 97.9% when the 24-h BMD result was used as a reference to 93.7% when the 48-h BMD result was used as reference (95.2 and 83.0%, respectively, for on-scale results). Of the discrepancies noted between the VITEK 2 and 24-h BMD MIC results, the MICs generated by the VITEK 2 were higher than those obtained by BMD in all nine instances (100%). In contrast, of the 27 discrepancies observed between THE VITEK 2 and 48-h BMD MIC results, the MICs generated by the VITEK 2 were lower than those obtained by BMD in 21 instances (77.8%). The latter discrepancies occurred almost exclusively with *C. glabrata* and *C. krusei*, emphasizing the impact of trailing on BMD results obtained with these two species.

The mean time to result for the VITEK 2 system was 13 h, with a range from 10 to 26 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 EA between the VITEK 2 results and either the 24-h or the 48-h BMD MICs was >95% for all species with the exception of *C. krusei*. Whereas the EA for this species was 100% when VITEK 2 results were compared to the 24-h BMD MICs, it was only 68.6% when the 48-h BMD MICs were used as the reference result. Again, these discrepancies were due to higher MICs obtained with the BMD method than were obtained with the VITEK 2 system for this species. Given the CLSI recom-

TABLE 1. Fluconazole susceptibilities of 426 isolates of *Candida* spp. as determined by the VITEK 2 yeast susceptibility test and by CLSI BMD methods[a]

Species	No. tested	Test method[b]	MIC (μg/ml)[c]			EA (%)[d]
			Range	50%	90%	
C. albicans	198	VITEK 2	≤1–≥64	≤1	≤1	
		BMD-24	≤0.12–≥128	0.25	0.5	97.0
		BMD-48	≤0.12–≥128	0.25	0.5	96.5
C. dubliniensis	8	VITEK 2	≤1–32	≤1		
		BMD-24	≤0.12–16	≤0.12		100.0
		BMD-48	≤0.12–16	0.25		100.0
C. glabrata	83	VITEK 2	≤1–≥64	8	≥64	
		BMD-24	0.5–≥128	4	64	98.8
		BMD-48	0.5–≥128	16	≥128	96.4
C. guilliermondii	3	VITEK 2	≤1–4	2		
		BMD-24	1–4	4		100.0
		BMD-48	2–4	4		100.0
C. krusei	51	VITEK 2	≤1–≥64	16	32	
		BMD-24	≤1–≥128	16	32	100.0
		BMD-48	≤1–≥128	64	≥128	68.6
C. lusitaniae	9	VITEK 2	≤1–2	1		
		BMD-24	≤0.12–1	0.5		100.0
		BMD-48	≤0.12–2	0.5		100.0
C. norvegensis	1	VITEK 2	8			
		BMD-24	16			100.0
		BMD-48	16			100.0
C. parapsilosis	43	VITEK 2	≤1–≥64	≤1	2	
		BMD-24	0.25–4	0.5	1	95.3
		BMD-48	0.5–4	1	2	97.7
C. pelliculosa	2	VITEK 2	2–4	2		
		BMD-24	2–4	2		100.0
		BMD-48	4	4		100.0
C. tropicalis	28	VITEK 2	≤1–8	≤1	≤1	
		BMD-24	≤0.12–8	0.25	1	100.0
		BMD-48	0.25–16	0.5	4	100.0
All *Candida* spp.	426	VITEK 2	≤1–≥64	≤1	16	
		BMD-24	≤0.12–≥128	0.5	16	97.9
		BMD-48	≤0.12–≥128	0.5	64	93.7

[a] Isolates included both clinical ($n = 346$) and challenge ($n = 80$) sets.
[b] BMD-24 and BMD-48, BMD performed at 24 h and 48 h of incubation, respectively.
[c] 50% and 90%, MICs encompassing 50% and 90% of isolates tested, respectively.
[d] % EA is the EA (±2 $\log_2$ dilutions) between VITEK 2 and BMD MICs.

mendation that *C. krusei* should be considered resistant to fluconazole irrespective of the MIC, these discrepancies should not pose a problem clinically (20).

The VITEK 2 fluconazole MIC results were highly reproducible, as determined by replicate testing of a panel of 10 *Candida* spp. isolates in the three laboratories (Table 2). Both intra- and interlaboratory reproducibility was 100% for all 10 organisms. This high level of reproducibility underscores the excellent level of test standardization achieved with this automated microbiology system.

CA between the VITEK 2 and BMD methods was assessed by combining the data obtained with the clinical and challenge organism collections in all three laboratories (Table 3). Excellent CA was observed for all comparisons with the exception of *C. glabrata* and *C. norvegensis*. The overall CA for the comparison of VITEK 2 results with 24-h BMD results was 97.2% with no very major or major errors. Only *C. glabrata* (89.2%) and *C. norvegensis* (0.0%) among the 10 species tested showed less than 90% absolute CA with the 24-h BMD results. All of the errors observed with *C. glabrata* were minor errors and were the result of isolates determined to be susceptible by BMD and SDD by VITEK 2. This is not surprising given the tendency of fluconazole MICs for this species to fall close to the susceptible breakpoint: 82% of MICs determined by VITEK 2 and 78% of MICs determined by 24-h BMD fell between 4 and 16 μg/ml (data not shown). Importantly, all isolates of *C. glabrata* testing as resistant by the BMD method at 24 h were also

TABLE 2. VITEK 2 Fluconazole MIC reproducibility within and among three different laboratories

Species	Sample	Study site	No. of results at an MIC (μg/ml) of:						
			≤1	2	4	8	16	32	≥64
C. glabrata	304201	1				7	2		
		2				9			
		3				8	1		
		All				24	3		
C. glabrata	304927	1			1	8			
		2				9			
		3				9			
		All			1	26			
C. haemulonii	304848	1				1	7	1	
		2					6	3	
		3					7	2	
		All				1	20	6	
C. krusei	304204	1				8	1		
		2				8	1		
		3				7	2		
		All				23	4		
C. krusei	304845	1					8	1	
		2					8	1	
		3					4	5	
		All					20	7	
C. krusei	304850	1					8	1	
		2					9		
		3					7	2	
		All					24	3	
C. lipolytica	304856	1			9				
		2			5	4			
		3		1	8				
		All		1	22	4			
C. lusitaniae	304205	1		9					
		2		9					
		3		9					
		All		27					
C. norvegensis	304852	1						9	
		2						8	1
		3						9	
		All						26	1
C. pelliculosa	304847	1		4	5				
		2		3	6				
		3		4	5				
		All		11	16				

resistant with the VITEK 2 system. Only one isolate of *C. norvegensis* was tested and was found to be susceptible by VITEK 2 (MIC = 8 μg/ml) and SDD by 24-h BMD (MIC = 16 μg/ml).

Although the overall CA between VITEK 2 and the 48-h BMD results was lower than that seen with the 24-h BMD comparison (88.3% versus 97.2%), the vast majority of errors were minor, and almost all were due to a shift in the MICs for *C. glabrata* from susceptible at 24 h to SDD at 48 h with the BMD method (Table 3). There were no ME and only one VME (0.2% of all isolates and 1.5% of all resistant isolates), the latter being a *C. glabrata* result. Again, with the exception of *C. glabrata* and *C. norvegensis*, the CA between the VITEK 2 and 48-h BMD results exceeded 95% for all species of *Candida*. All but one of the errors observed with *C. glabrata* were minor and can be attributed to clustering of fluconazole MICs around the S-SDD breakpoint (66% of the 48-h BMD results fell in the range from 4 to 16 μg/ml, and 75% were in the range 8 to 32 μg/ml) and the influence of trailing growth seen with this species and fluconazole.

The overall pattern of results shown in Table 3 was also seen with clinical isolates tested in each of the three laboratories (Table 4). In each laboratory the categorical agreement was ≥95% when VITEK 2 results were compared to 24-h BMD results and ranged from 88.0 to 89.6% with the comparison between VITEK 2 and 48-h BMD. A shift from S to SDD was observed in all three laboratories when 24-h BMD results were compared to 48-h BMD results.

The findings of the present study document the excellent degree of standardization and reproducibility that can be achieved with the VITEK 2 yeast susceptibility test. This system is the first automated approach to antifungal susceptibility testing and as such provides the ultimate in test standardization. In addition to providing highly reproducible results, the VITEK 2 system was rapid, with a mean time to result of 13 h. The availability of rapid quantitative antifungal susceptibility data will be a major step in optimizing the therapy of invasive candidal infections (1, 13, 15, 18).

The EA observed in the present study between VITEK 2 and BMD MICs demonstrates excellent quantitative agreement between the methods for all species of *Candida* tested and is comparable or superior to that reported for other commercial systems (8–11, 16, 17, 24, 25). The highly automated, hands-off nature of the system virtually eliminates the subjectivity that affects all other test systems.

The use of a spectrophotometer to provide objective, and earlier, readings of MICs is well established (8, 11, 12, 16). It is also proposed as a means by which one can mitigate falsely elevated fluconazole MICs due to trailing growth (2, 7, 8, 11, 12). Based on the comparative data presented herein, it appears that the VITEK 2 yeast susceptibility test accomplishes these objectives as well. Thus, although the CA between the VITEK 2 and the 48-h BMD results was lower than that observed for the 24-h BMD comparison, it may be the latter that is more relevant clinically (2, 11, 16). Earlier work has shown that the 24-h fluconazole MIC endpoint correlated better than the 48-h endpoint with sterol quantification (2) and with treatment outcome both clinically (29) and in a murine model of invasive candidiasis (31). These findings suggest that fluconazole results for isolates of *Candida* spp. with significant trailing should be interpreted on the basis of the lower MIC observed at the earlier (24-h) time point (16, 20). Given the evidence of trailing, especially with *C. glabrata* and *C. krusei*, at 48 h shown in Tables 1 and 3, the most appropriate comparator for the VITEK 2 may be the 24-h BMD results. This comparison indicates that the VITEK 2 provides highly accurate quantitative and qualitative results for fluconazole and *Candida* spp.

The value of a rapid, automated, commercially available antifungal susceptibility testing system such as the VITEK 2 is not limited to the generation of fluconazole susceptibility data. Recently, we have shown that fluconazole may serve as a sur-

TABLE 3. Categorical agreement between VITEK 2 yeast susceptibility test MICs and 24-h and 48-h CLSI BMD fluconazole MICs for 426 isolates of *Candida* spp. in three laboratories[a]

Species (no. of strains tested)	Test method	% of MICs by category			% CA	% Errors		
		S	SDD	R		VME	ME	Minor
C. albicans (198)	VITEK 2	97.5	1.0	1.5				
	BMD-24	98.0	0.5	1.5	99.5	0.0	0.0	0.5
	BMD-48	97.5	1.0	1.5	99.0	0.0	0.0	1.0
C. dubliniensis (8)	VITEK 2	75.0	25.0	0.0				
	BMD-24	75.0	25.0	0.0	100.0	0.0	0.0	0.0
	BMD-48	75.0	25.0	0.0	100.0	0.0	0.0	0.0
C. glabrata (83)	VITEK 2	75.9	13.3	10.8				
	BMD-24	84.3	4.8	10.9	89.2	0.0	0.0	10.8
	BMD-48	27.7	59.0	13.3	45.8	1.2	0.0	53.0
C. guilliermondii (3)	VITEK 2	100.0	0.0	0.0				
	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. krusei[b] (51)	VITEK 2	0.0	0.0	100.0				
	BMD-24	0.0	0.0	100.0	100.0	0.0	0.0	0.0
	BMD-48	0.0	0.0	100.0	100.0	0.0	0.0	0.0
C. lusitaniae (9)	VITEK 2	100.0	0.0	0.0				
	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. norvegensis (1)	VITEK 2	100.0	0.0	0.0				
	BMD-24	0.0	100.0	0.0	0.0	0.0	0.0	100.0
	BMD-48	0.0	100.0	0.0	0.0	0.0	0.0	100.0
C. parapsilosis (43)	VITEK 2	97.7	2.3	0.0				
	BMD-24	100.0	0.0	0.0	97.7	0.0	0.0	2.3
	BMD-48	100.0	0.0	0.0	97.7	0.0	0.0	2.3
C. pelliculosa (2)	VITEK 2	100.0	0.0	0.0				
	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	BMD-48	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. tropicalis (28)	VITEK 2	100.0	0.0	0.0				
	BMD-24	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	BMD-48	96.4	3.6	0.0	96.4	0.0	0.0	3.6
All *Candida* spp. (426)	VITEK 2	81.5	3.8	14.7				
	BMD-24	83.3	2.0	14.7	97.2	0.0	0.0	2.8
	BMD-48	71.8	12.9	15.3	88.3	0.2	0.0	11.5

[a] Isolates include both clinical ($n = 346$) and challenge ($n = 80$) sets.
[b] *C. krusei* categorical results forced to R regardless of MICs.

TABLE 4. Agreement between VITEK 2 yeast susceptibility test and reference BMD MIC results for fluconazole against clinical isolates of *Candida* spp. in each of three laboratories

Study site (no. of strains tested)	BMD incubation time (h)	% of BMD by category			% CA	% Errors		
		S	SDD	R		VME	ME	Minor
1 (135)	24	86.0	0.7	13.3	98.5	0.0	0.0	1.5
	48	75.6	11.1	13.3	89.6	0.0	0.0	10.4
2 (108)	24	81.5	0.9	17.6	97.2	0.0	0.0	2.8
	48	69.4	11.1	19.5	88.0	0.9	0.0	11.1
3 (103)	24	79.6	2.9	17.5	95.1	0.0	0.0	4.9
	48	70.0	12.5	17.5	89.3	0.0	0.0	10.7

rogate marker for voriconazole susceptibility of *Candida* spp. (28). Specifically, fluconazole MICs of ≤32 μg/ml predict susceptibility and MICs of ≥64 μg/ml predict resistance of *Candida* spp. to voriconazole with an absolute CA of 97%, 0.1% VME, and 1.4% ME. Thus, clinical laboratories performing antifungal susceptibility testing of fluconazole using the VITEK 2 can reliably use these results as surrogate markers of susceptibility and resistance to voriconazole until commercial FDA-approved voriconazole susceptibility tests become available.

In summary, the MICs of fluconazole can be determined in an automated fashion in less than 15 h for most species of *Candida* with the VITEK 2 yeast susceptibility test. The VITEK 2 system ensures that each test is performed in a highly standardized manner and provides quantitative MIC results that are reproducible and accurate. The use of spectrophotom-

etry to determine the MIC endpoint eliminates subjectivity and minimizes the effect of trailing growth that compromises the performance of systems relying on visual MIC determination. The VITEK 2 system reliably identifies fluconazole resistance among *Candida* spp. and demonstrates excellent quantitative and qualitative agreement with the reference BMD method. The introduction of the VITEK 2 system in the clinical laboratory will be an important step toward the optimization of antifungal therapy of candidiasis.

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