

# 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 Yeasts and Yeast-Like Organisms

A. ESPINEL-INGROFF,<sup>1\*</sup> M. PFALLER,<sup>2</sup> S. A. MESSER,<sup>2</sup> C. C. KNAPP,<sup>3</sup> S. KILLIAN,<sup>3</sup>  
H. A. NORRIS,<sup>4</sup> AND M. A. GHANNOUM<sup>4</sup>

Medical College of Virginia of Virginia Commonwealth University, Richmond, Virginia<sup>1</sup>; University of Iowa College of Medicine, Iowa City, Iowa<sup>2</sup>; and AccuMed International, Westlake,<sup>3</sup> and University Hospital of Cleveland and Case Western Reserve University, Cleveland,<sup>4</sup> Ohio

Received 21 September 1998/Returned for modification 21 October 1998/Accepted 11 December 1998

National Committee for Clinical Laboratory Standards (NCCLS) standard guidelines are available for the antifungal susceptibility testing of common *Candida* spp. and *Cryptococcus neoformans*, but NCCLS methods may not be the most efficient and convenient procedures for use in the clinical laboratory. MICs of amphotericin B, fluconazole, flucytosine, itraconazole, and ketoconazole were determined by the commercially prepared Sensititre YeastOne Colorimetric Antifungal Panel and by the NCCLS M27-A broth microdilution method for 1,176 clinical isolates of yeasts and yeast-like organisms, including *Blastoschizomyces capitatus*, *Cryptococcus* spp., 14 common and emerging species of *Candida*, *Hansenula anomala*, *Rhodotorula* spp., *Saccharomyces cerevisiae*, *Sporobolomyces salmonicolor*, and *Trichosporon beigeli*. Colorimetric MICs of amphotericin B corresponded to the first blue well (no growth), and MICs of the other agents corresponded to the first purple or blue well. Three comparisons of MIC pairs by the two methods were evaluated to obtain percentages of agreement: 24- and 48-h MICs and 24-h colorimetric versus 48-h reference MICs. The best performance of the YeastOne panel was with 24-h MICs (92 to 100%) with the azoles and flucytosine for all the species tested, with the exception of *C. albicans* (87 to 90%). For amphotericin B, the best agreement between the methods was with 48-h MIC pairs (92 to 99%) for most of the species tested. The exception was for isolates of *C. neoformans* (76%). These data suggest the potential value of the YeastOne panel for use in the clinical laboratory.

In 1997, the National Committee for Clinical Laboratory Standards (NCCLS) published standardized broth macro- and microdilution methods (NCCLS document M27-A) for antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* (6). These standard guidelines also are being applied for a variety of emerging yeasts and yeast-like organisms. However, the NCCLS methods may not be the most efficient and convenient procedures for use in the clinical laboratory. Alternative approaches that are more convenient and efficient are needed as the demand for in vitro antifungal data continues to increase, although commercial development of such systems has been slow. The Sensititre YeastOne Colorimetric Antifungal Panel (AccuMed International, Westlake, Ohio) consists of a disposable tray which contains dried serial dilutions of five established antifungal agents in individual wells. The wells also contain the color indicator Alamar Blue (AccuMed International). Earlier comparisons of NCCLS and colorimetric MIC data by using Alamar Blue as the colorimetric indicator have shown favorable results (7–9, 15, 16), and a prior investigation regarding the reproducibility of MIC endpoints among three laboratories by using the YeastOne panel

has demonstrated a high degree of intra- and interlaboratory reproducibility for a set of 10 isolates of *Candida* spp. (12).

The purpose of this study was to evaluate the performance of the YeastOne panel in three independent laboratories against a set of 1,176 clinical isolates of yeasts and yeast-like organisms, including *Blastoschizomyces capitatus*, 14 common and emerging species of *Candida*, and 5 species of *Cryptococcus*, as well as 5 species of other emerging yeast and yeast-like pathogens. Colorimetric MICs of the five agents were compared to M27-A MICs at 24 and 48 h (>48 h for *Cryptococcus* spp. and some other species) for each isolate-drug combination.

## MATERIALS AND METHODS

**Study design.** The study was designed to compare MICs obtained by the YeastOne panel to those obtained by the M27-A broth microdilution method (6) in the three independent laboratories (each laboratory tested by each method and with the five antifungal agents one-third of the total number of 1,176 isolates evaluated). Two MIC readings were performed by each method, e.g., 24 and 48 h, for most yeasts and yeast-like organisms tested, with the exception of *Cryptococcus* spp. and some of the emerging yeast pathogens (MICs for these organisms were read at 48 to 96 h). Each first and second day, colorimetric MICs for each isolate-drug combination were compared to both corresponding first- and second-day M27-A MICs.

**Clinical isolates.** A total of 1,176 clinical isolates from the culture collections of the University of Iowa College of Medicine, the Medical College of Virginia of Virginia Commonwealth University, and the University Hospital of Cleveland were used. These included 896 common *Candida* spp. (468 *C. albicans*, 95 *C. glabrata*, 67 *C. krusei*, 77 *C. lusitanae*, 95 *C. parapsilosis*, and 94 *C. tropicalis* isolates), 84 emerging *Candida* spp. (*C. ciferrii*, *C. famata*, *C. guilliermondii*, *C. lambica*, *C. lipolytica*, *C. rugosa*, and *C. zeylanoides* isolates), 13 *Blastoschizomyces*

\* Corresponding author. Mailing address: Medical College of Virginia of Virginia Commonwealth University, P.O. Box 980049, Richmond, VA 23298-0049. Phone: (804) 828-9711. Fax: (804) 828-3097. E-mail: AVINGROFF@HSC.VCU.EDU.

*capitatus* isolates, 11 *Hansenula anomala* isolates, 10 *Rhodotorula* spp., 23 *Saccharomyces cerevisiae* isolates, 2 *Sporobolomyces salmonicolor* isolates, 19 *Trichosporon beigeli* isolates, 107 *C. neoformans* isolates (Table 1), and 11 non-*C. neoformans* *Cryptococcus* isolates (*C. albidus*, *C. laurentii*, *C. terreus*, and *C. uniguttulatus* isolates). The NCCLS quality control (QC) isolates, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, also were tested each time a set of clinical isolates was evaluated by the two procedures. The clinical isolates were recovered from either oral cavities, blood, or other body fluids. Each isolate represented a unique strain from a single patient managed in one of several medical centers in the United States and Europe. The set of isolates included strains with different patterns of susceptibility to fluconazole and itraconazole, that is, resistant, susceptible dose-dependent (S-DD), and susceptible isolates of the common *Candida* spp. (Table 2) (6). Yeast isolates were maintained in sterile water and subcultured on antimicrobial-free medium to ensure viability and purity prior to testing.

**Antifungal agents.** The YeastOne panels and microdilution trays containing serial dilutions of amphotericin B, fluconazole, flucytosine, itraconazole, and ketoconazole were provided by AccuMed International. Amphotericin B, itraconazole, and ketoconazole dilutions ranged from 16 to 0.008  $\mu\text{g/ml}$  and fluconazole and flucytosine dilutions ranged from 64 to 0.03  $\mu\text{g/ml}$  in the M27-A microdilution trays and YeastOne panels. The YeastOne panels were shipped in sealed packages and were stored at ambient temperature until testing was performed. The microdilution trays, which were prepared by following the M27-A additive procedure (6), were shipped frozen to each participant laboratory and stored at  $-70^{\circ}\text{C}$  until the day of the test.

**Inoculum preparation.** Stock inoculum suspensions of the yeasts were obtained from 24-h cultures (48 h for *Cryptococcus* spp.) on Sabouraud dextrose agar at  $35^{\circ}\text{C}$ . The turbidity of each yeast suspension was adapted by the spectrophotometric method by following the M27-A guidelines (6).

**Sensititre YeastOne Colorimetric Antifungal Panel procedure.** On the day of the test, a working yeast suspension of approximately  $1.5 \times 10^3$  CFU/ml was prepared in YeastOne broth (AccuMed International). The dried YeastOne panels were rehydrated with the working yeast suspension using a multichannel pipetting device by dispensing 100  $\mu\text{l}$  into each well. The YeastOne panels were covered with seal strips and incubated at  $35^{\circ}\text{C}$  for 24 to 96 h in a non- $\text{CO}_2$  incubator and were read either after 24 and 48 h (most *Candida* spp. and other yeasts and yeast-like organisms) or after 48, 72, and 96 h (*Cryptococcus* spp. and certain emerging yeasts and yeast-like organisms) of incubation by using a view box and normal laboratory lighting. If the isolate did not grow at  $35^{\circ}\text{C}$ , the test was repeated and the panel was incubated at  $30^{\circ}\text{C}$ . Yeast growth in the colorimetric antifungal solutions was evident as a change in the growth indicator from blue (negative) to red or purple (positive). Colorimetric MICs were interpreted as the lowest concentration of antifungal solutions changing from red (growth) to blue (no growth) (amphotericin B) or changing from red to purple (growth inhibition) or to blue (no growth) (azoles and flucytosine). Both QC isolates were tested in the same manner each day a set of isolates were evaluated in each participant laboratory.

**NCCLS broth microdilution method (M27-A).** The stock inoculum suspensions were prepared as for the YeastOne panel and the adjusted suspension was diluted 1:1,000 in standard RPMI-1640 with 1.5% dextrose, which resulted in  $2 \times$  the final test concentration of  $0.5 \times 10^3$  to  $2.5 \times 10^3$  CFU/ml as recommended in the NCCLS M27-A document (6). On the day of the test, each well was inoculated with 100  $\mu\text{l}$  of the corresponding diluted  $2 \times$  inoculum suspension. The microdilution trays were incubated at  $35^{\circ}\text{C}$  for 24 to 96 h in a non- $\text{CO}_2$  incubator. If the isolate did not grow at  $35^{\circ}\text{C}$ , the test was repeated and the microdilution tray was incubated at  $30^{\circ}\text{C}$ . MICs were determined either after 24 and 48 h (*Candida* spp. and most other yeasts and yeast-like organisms) or after 48 and 72 h (*Cryptococcus* spp. and certain emerging yeasts and yeast-like organisms) or after 48, 72, and 96 h (*Cryptococcus* spp. and certain emerging yeasts and yeast-like organisms) of incubation by comparing the growth in each MIC well to the growth in the control well (drug-free medium) with the aid of a reading mirror. Reference MICs corresponded to the lowest drug dilution that showed complete inhibition (amphotericin B) and the lowest drug dilution that showed prominent growth inhibition (50% or more [the azoles and flucytosine]). Both QC isolates were tested in the same manner each day a set of isolates were evaluated in each participant laboratory.

**Data analysis.** MICs for each drug-organism combination by each method obtained in the three laboratories were compared as follows: (i) 24-h MIC pairs by the two methods, (ii) 24-h colorimetric MIC versus M27-A 48-h MIC, and (iii) 48-h MIC pairs by the two methods. Both on-scale (e.g., 0.12 and 64  $\mu\text{g/ml}$ ) and off-scale (e.g.,  $<0.12$  and  $>64$   $\mu\text{g/ml}$ ) MICs were included in the analysis. Discrepancies between MIC pairs of no more than 3 dilutions (3 wells, e.g., 0.5, 1.0, and 2  $\mu\text{g/ml}$ ) were used for calculations of percent agreement. The percentage of MIC endpoints within 3 dilutions between the two methods was then determined for each combination of isolate, drug, and incubation time.

## RESULTS

Lack of growth precluded MIC determination by the YeastOne panel for 13 (1.5%) of the 896 isolates grouped as common *Candida* spp. and for 30 (19%) of the 162 isolates grouped

as emerging *Candida* spp. and yeasts and yeast-like organisms (mostly *Candida* spp., *H. anomala*, *Rhodotorula* spp., and *S. salmonicolor* isolates) and by the reference method for 14 (1.6%) isolates of the common *Candida* spp. and for 21 (25%) isolates of emerging *Candida* spp. MICs for most isolates of *Rhodotorula* spp. and for the two *S. salmonicolor* isolates were determined only at 72 h due to lack of growth at 24 and 48 h. In addition, five of the seven *C. lambica* isolates tested did not grow (96 h) in either the YeastOne panel or the NCCLS microdilution trays. The same was observed with one of the nine *C. lipolytica* isolates and one of the seven *C. zeylanoides* isolates tested by the YeastOne panel. As expected, only 4 of the 11 non-*C. neoformans* *Cryptococcus* isolates tested grew at  $35^{\circ}\text{C}$ ; MIC data were obtained for 4 of the remaining 7 isolates at  $30^{\circ}\text{C}$  after 72 h of incubation by the M27-A method and after 96 h with the YeastOne panel.

The results in Table 1 are based on the actual number of isolates for which MICs were determined at the listed incubation times; values for the common *Candida* spp. represent the percent agreement ranges for the five species in this group. As summarized in Table 1, the performance of the YeastOne panel was dependent to a certain degree on the species, antifungal agent, and (especially) length of incubation. For amphotericin B, the best agreement (90 to 99%) was seen after 48 h of incubation for most of the species tested. The exception was for isolates of *C. neoformans* (76%), where colorimetric MICs were 3 to 5 dilutions lower for 25 of the 107 isolates tested. On the other hand, the agreement between the two methods with the other drugs was good (90 to 98%) for this species. When MIC data were obtained at either 30 or  $35^{\circ}\text{C}$  for eight non-*C. neoformans* *Cryptococcus* isolates, agreement between the two methods was observed for seven of these isolates with the azoles and flucytosine and for eight isolates with amphotericin B (data not shown in Table 1).

For isolates of *C. albicans*, the agreement was higher (87 to 90%) between 24-h MICs of the azoles and flucytosine by the two methods than between the other two sets (24 versus 48 h and 48 versus 48 h) of MIC pairs (54 to 89%; Table 1). The main reason for this was that colorimetric MICs were consistently more than 2 dilutions higher after 48 h of incubation than reference MICs, especially for isolates of *C. albicans* recovered from patients with oropharyngeal infections. The performance of the YeastOne panel with the azoles and flucytosine against *C. glabrata* and *C. tropicalis* was also superior with 24-h (90 to 99%) than with 48-h (66 to 89%) MICs (data not shown in Table 1). The agreement between the methods for *C. krusei*, *C. lusitanae*, and *C. parapsilosis* was less dependent on the incubation time, with 95 to 99% agreement between 24-h colorimetric and 48-h reference MICs and 90 to 100% agreement between the other two sets of MIC pairs (data not shown in Table 1).

For emerging *Candida* spp. and other yeasts and yeast-like species, the percentages of agreement between the two procedures were also less dependent on the incubation time; agreement between the two methods was good (90%) to excellent (100%) with all 24- and 48-h MIC pairs of the five antifungal agents (Table 1). However, as stated above, many of these isolates required more than 24 h of incubation to show sufficient growth for MIC determination, especially isolates of *H. anomala*, *Rhodotorula* spp., *S. salmonicolor*, and most strains of emerging *Candida* spp. (e.g., *C. ciferrii*, *C. guilliermondii*, *C. lambica*, *C. lipolytica*, and *C. zeylanoides*).

Table 2 shows the percent agreement between the two methods regarding the ranking of isolates within the three categories of interpretive breakpoints that have been recently established by the NCCLS for fluconazole, flucytosine, and

TABLE 1. Percent agreement between colorimetric and NCCLS reference broth microdilution MIC pairs<sup>a</sup>

Fungus (no. of isolates tested)	Antifungal agent	% Agreement (range) between MIC pairs at incubation time (h)		
		24 vs 24	48 vs 48	24 vs 48
<i>C. albicans</i> (465)	Amphotericin B	97	97	76
	Fluconazole	87	60	71
	Flucytosine	87	56	89
	Itraconazole	89	54	76
	Ketoconazole	90	56	69
Other common <i>Candida</i> spp. (417) <sup>b</sup>	Amphotericin B	85–97	92–99	40–75
	Fluconazole	95–98	73–99	91–99
	Flucytosine	92–99	73–100	89–97
	Itraconazole	93–100	66–97	86–97
	Ketoconazole	93–100	66–97	85–97
Emerging <i>Candida</i> spp. (64) <sup>c</sup>	Amphotericin B	90	90	63
	Fluconazole	98	94	97
	Flucytosine	95	99	77
	Itraconazole	92	91	90
	Ketoconazole	95	94	85
<i>C. neoformans</i> (107) <sup>d</sup>	Amphotericin B	ND	76	ND
	Fluconazole	ND	98	ND
	Flucytosine	ND	96	ND
	Itraconazole	ND	96	ND
	Ketoconazole	ND	90	ND
Miscellaneous yeasts and yeast-like organisms (68) <sup>e</sup>	Amphotericin B	91	99	67
	Fluconazole	100	99	99
	Flucytosine	99	99	94
	Itraconazole	97	95	90
	Ketoconazole	94	97	90

<sup>a</sup> Sensititre YeastOne Colorimetric Antifungal Panel MICs versus NCCLS broth microdilution M27-A MICs. An incubation time of 48 to 96 h was used for *C. neoformans* and certain emerging yeast pathogens. ND, not determined.

<sup>b</sup> Species included 77 to 95 isolates each of *C. glabrata*, *C. krusei*, *C. lusitanae*, *C. parapsilosis*, and *C. tropicalis*.

<sup>c</sup> Species included 5 to 26 isolates each of *C. ciferrii*, *C. famata*, *C. guilliermondii*, *C. kefyr*, *C. lambica*, *C. lypolytica*, *C. rugosa*, and *C. zeylanoides*.

<sup>d</sup> The eight non-*C. neoformans* *Cryptococcus* isolates tested are not listed.

<sup>e</sup> Species included 2 to 23 isolates each of *B. capitatus*, *H. anomala*, *R. rubra*, *R. minuta*, *S. cerevisiae*, *S. salmonicolor*, and *T. beigelii*.

itraconazole (6). Interpretive breakpoints have not been established for either amphotericin B or ketoconazole against any fungal species or for any antifungal agent against *C. neoformans*, other *Cryptococcus* spp., or the various yeasts and yeast-like organisms evaluated in this study. For this reason and

because the best overall performance of the YeastOne panel was after 24 h of incubation, only the agreements for 24-h colorimetric data for the common *Candida* spp. were evaluated (Table 2). Overall, the performance of the YeastOne panel was good (86%) to excellent (>90%) for most species-drug com-

TABLE 2. Percent agreement between YeastOne panel and NCCLS reference method for ranking isolates of common *Candida* spp. within the established breakpoints<sup>a</sup>

Antifungal agent	Category and breakpoint (μg/ml) <sup>b</sup>	% Agreement by species (no. of isolates at each breakpoint)					
		<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>C. lusitanae</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>
Fluconazole	S (≤8)	89 (442)	96 (49)	NA <sup>c</sup>	96 (76)	98 (87)	95 (89)
	S-DD (16–32)	75 (12)	94 (32)	NA	ND (0)	ND (7)	ND (2)
	R (≥64)	93 (14)	100 (14)	NA	ND (1)	ND (1)	ND (3)
Itraconazole	S (≤0.12)	91 (346)	ND (5)	ND (6)	93 (30)	100 (38)	100 (39)
	S-DD (0.2–0.5)	24 (104)	97 (40)	100 (49)	98 (43)	100 (54)	89 (48)
	R (≥1.0)	50 (18)	90 (48)	100 (12)	ND (4)	ND (3)	ND (7)
Flucytosine	S (≤4)	88 (446)	98 (93)	91 (23)	98 (64)	99 (95)	95 (88)
	I (8–16)	ND (2)	ND (0)	100 (44)	ND (1)	ND (0)	ND (2)
	R (≥32)	75 (20)	ND (2)	ND (0)	100 (12)	ND (0)	ND (4)

<sup>a</sup> MICs were read at 24 h for both methods. ND, not determined.

<sup>b</sup> NCCLS breakpoints: S, susceptible; S-DD, susceptible-dose dependent; I, intermediate; R, resistant.

<sup>c</sup> NA, not applicable: the breakpoints do not apply to *C. krusei* and fluconazole (intrinsically resistant species).

binations. The most consistent exceptions were among itraconazole-resistant and S-DD isolates of *C. albicans*. The reference MICs for the 104 S-DD isolates were 0.2 to 0.5  $\mu\text{g/ml}$ , whereas the colorimetric MICs were 0.03 to 0.12  $\mu\text{g/ml}$  for 58 of these isolates and 1 to 16  $\mu\text{g/ml}$  for an additional 18 isolates. The MIC for 9 of the 18 resistant isolates was 0.5  $\mu\text{g/ml}$  in the colorimetric system instead of  $\geq 1.0$   $\mu\text{g/ml}$ . These are considered minor errors. Therefore, the differences between colorimetric and reference MICs for 63% of these isolates were within the 3-dilution range allowed for MIC comparisons. Although percentages were not obtained when less than 10 isolates were ranked by the reference method within each breakpoint, colorimetric MICs for those few isolates were usually categorized within the expected breakpoint.

## DISCUSSION

There are two commercially available antifungal susceptibility testing systems for fungal pathogens in the United States, the E test (1, 3, 10) and the YeastOne panel (12), but these methods are available only for investigational purposes pending Food and Drug Administration approval. The performance of NCCLS-based colorimetric broth microdilution methods, using the same Alamar Blue indicator as in the YeastOne panel, has been comparable to that of the NCCLS M27-A broth macro- and microdilution methods in several studies (7–9, 15, 16). In the present study the performance of the YeastOne panel was to certain degree dependent on the species tested, the antifungal agent evaluated, and the incubation time.

One of the drawbacks of the standard RPMI medium is its inability to yield sufficient growth before 72 h of incubation for antifungal susceptibility testing of *C. neoformans*. The YeastOne panel was designed for shorter incubation times (24 to 48 h) because its colorimetric indicator, Alamar Blue, is not as stable after longer incubation times. However, the lower level of agreement observed between amphotericin B reference and colorimetric MICs for *C. neoformans* could not be attributed to lack of indicator stability. Good to excellent agreement (90 to 98%) was demonstrated with the other four antifungal agents as well as with amphotericin B in one of the three testing centers for this species. In addition, since colorimetric amphotericin B MICs were lower, not higher, than the reference MICs, there is not a logical explanation for this phenomenon. Prior comparative studies of antifungal MIC data obtained either by two methods or by the same methods among different laboratories, including evaluations of MICs for *C. neoformans*, have resulted in superior or similar (91 to 100%) agreement for amphotericin B relative to the agreement for the other agents (67 to 100%) (2, 11). Comparisons involving colorimetric MICs for *C. neoformans* obtained by using Alamar Blue as the indicator have resulted in lower (40%) agreement with the NCCLS macrodilution method (16) but in higher agreement (99%) with the NCCLS microdilution method (7) than those obtained in our study (76%). It would be worthwhile to further evaluate the performance of the YeastOne panel by using yeast nitrogen base medium instead of the standard RPMI-1640 for testing isolates of *C. neoformans*. This medium has been shown to support better growth of this species for the determination of fluconazole MICs (14). The results of the present study with amphotericin B for the common *Candida* spp. (92 to 99% agreement) are similar to those previously reported (90 to 99% agreement) (7). Colorimetric amphotericin B MIC data are not available for most of the other yeast and yeast-like pathogens tested, and little data are available even by the NCCLS or other methods for these species (4, 5).

Colorimetric MICs of the azoles and flucytosine for *C. albicans* were consistently higher after 48 h than after 24 h of incubation. Since reference MICs were usually the same at 24 and 48 h, a high degree of disagreement was observed between 48-h colorimetric and reference MICs. It has been demonstrated that variation can also be observed between 24- and 48-h (up to 128-fold higher) fluconazole MICs for a few strains of *C. albicans* by the NCCLS broth microdilution method and that the 24-h MIC better matched the in vivo response in a murine model of invasive candidiasis (13). Because of the heavier trailing seen after 48 than after 24 h of incubation, the 24-h result may be the most clinically useful azole MIC for most common *Candida* spp.

Earlier comparisons of fluconazole and flucytosine colorimetric MICs with NCCLS reference microdilution MICs for common *Candida* spp., including *C. albicans*, have also resulted in an overall superior agreement at 24 h (84 to 100%) than at 48 h (57 to 100%) (7). However, with the NCCLS macrodilution method, the agreement has been higher with 48-h (64 to 100%) than with 24-h (11 to 100%) colorimetric MICs (16). As in our study, the agreement has been species-drug combination and incubation time dependent in those studies, but our results are more consistent among the species with the optimal incubation time. In contrast, To and coinvestigators (16) reported that the colorimetric method was not a valid alternative for testing fluconazole against *C. tropicalis* and *C. glabrata* because only 11 to 71% of colorimetric and NCCLS MICs were in agreement. In this present study, major discrepancies were seen solely with 48-h colorimetric MICs. The colorimetric itraconazole MICs for *C. glabrata* were also more accurate in this study (94% agreement) than in a prior evaluation (86% agreement) (15).

Very little data have been published regarding the antifungal activities of either established or investigational agents against the organisms grouped in the present study as emerging *Candida* spp. and other yeast and yeast-like species. However, our colorimetric MIC data for these pathogens are similar to those previously obtained (4) by the NCCLS broth microdilution method. Although these and other emerging pathogens are not as frequently recovered from clinical isolates as the common *Candida* spp. and *C. neoformans*, the risk of opportunistic infections caused by emerging pathogens has increased in patients who are severely immunocompromised (5). Because MIC profiles are not available for some of these species, it is important to determine MIC data when one of these isolates is associated with a severe infection in an immunocompromised host. Testing conditions should be improved, because current methods do not yield adequate growth of certain emerging fungal pathogens and do not even yield sufficient growth at 30°C for non-*C. neoformans* *Cryptococcus* isolates.

In summary, our evaluation of the performance of the Sensititre YeastOne Colorimetric Antifungal Panel suggests its potential value for use in the clinical laboratory for the antifungal susceptibility testing of most *Candida* spp. and other yeasts and yeast-like organisms after 24 h of incubation and for *C. neoformans* after 72 h of incubation with fluconazole, flucytosine, itraconazole, and ketoconazole. On the other hand, determination of colorimetric amphotericin B MICs with the YeastOne panel should be obtained after 48 h of incubation.

## ACKNOWLEDGMENTS

Many thanks to Julie Rhodes for her secretarial assistance in preparation of the manuscript.

This study was partially supported by a grant from AccuMed International, Inc.

## REFERENCES

1. **Espinel-Ingroff, A.** 1994. Etest for antifungal susceptibility testing of yeasts. *Diagn. Microbiol. Infect. Dis.* **19**:217–220.
2. **Espinel-Ingroff, A., C. W. Kish, Jr., T. M. Kerkering, R. A. Fromtling, K. Bartizal, J. N. Galgiani, K. Villareal, M. Pfaller, T. Gerarden, M. G. Rinaldi, and A. Fothergill.** 1992. Collaborative comparison of broth macrodilution and microdilution antifungal susceptibility tests. *J. Clin. Microbiol.* **30**:3138–3145.
3. **Espinel-Ingroff, A., M. Pfaller, M. E. Erwin, and R. N. Jones.** 1996. Interlaboratory evaluation of the Etest method for antifungal susceptibility testing of pathogenic yeasts for five antifungal agents by using Casitone agar and solidified RPMI-1640 medium with 2% dextrose. *J. Clin. Microbiol.* **33**:535–540.
4. **Espinel-Ingroff, A.** 1998. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J. Clin. Microbiol.* **36**:198–202.
5. **Hazen, K.** 1995. New and emerging yeast pathogens. *Clin. Microbiol. Rev.* **8**:462–475.
6. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. M-27A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Pfaller, M. A., and A. L. Barry.** 1994. Evaluation of a novel colorimetric broth microdilution method for antifungal susceptibility testing of yeast isolates. *J. Clin. Microbiol.* **32**:1992–1996.
8. **Pfaller, M. A., B. Buschelman, M. Bale, M. Lancaster, A. Espinel-Ingroff, J. H. Rex, and M. Rinaldi.** 1994. Multicenter comparison of a colorimetric microdilution method with the reference macrodilution method for in vitro susceptibility testing of yeast isolates. *Diagn. Microbiol. Infect. Dis.* **19**:9–13.
9. **Pfaller, M. A., Q. Vu, M. Lancaster, A. Espinel-Ingroff, A. Fothergill, C. Grant, M. McGinnis, L. Pasarell, M. Rinaldi, and L. Steele-Moore.** 1994. Multisite reproducibility of colorimetric microdilution method for antifungal susceptibility testing of yeast isolates. *J. Clin. Microbiol.* **32**:1625–1628.
10. **Pfaller, M. A., S. A. Messer, A. Bolmstrom, F. C. Odds, and J. H. Rex.** 1996. Multicenter reproducibility of the Etest MIC method for antifungal susceptibility testing of yeast isolates. *J. Clin. Microbiol.* **34**:1691–1693.
11. **Pfaller, M. A., S. A. Messer, and S. Coffmann.** 1996. Comparison of visual and spectrophotometric methods of MIC endpoint determinations by using broth microdilution methods to test five antifungal agents, including the new triazole. *J. Clin. Microbiol.* **33**:1094–1097.
12. **Pfaller, M. A., S. A. Messer, R. J. Hollis, A. Espinel-Ingroff, M. A. Ghannoum, H. Plavan, S. Killian, and C. Knapp.** 1998. Multisite reproducibility of MIC results by the YeastOne colorimetric antifungal susceptibility panel. *Diagn. Microbiol. Infect. Dis.* **31**:543–547.
13. **Rex, J. H., P. W. Nelson, V. L. Paetznick, M. Lozano-Chiu, A. Espinel-Ingroff, and E. J. Anaissie.** 1998. Optimizing the correlation between results of testing in vitro and therapeutic outcome in vivo for fluconazole by testing critical isolates in a murine model of invasive candidiasis. *Antimicrob. Agents Chemother.* **42**:129–134.
14. **Sanati, H., S. A. Messer, M. Pfaller, M. Witt, R. Larsen, A. Espinel-Ingroff, and M. Ghannoum.** 1996. Multicenter evaluation of broth microdilution method for susceptibility testing of *Cryptococcus neoformans* against fluconazole. *J. Clin. Microbiol.* **34**:1280–1282.
15. **Tiballi, R. N., X. He, L. T. Zarins, S. G. Revankar, and C. A. Kauffman.** 1995. Use of a colorimetric system for yeast susceptibility testing. *J. Clin. Microbiol.* **33**:915–917.
16. **To, W.-K., A. W. Fothergill, and M. G. Rinaldi.** 1995. Comparative evaluation of macrodilution and Alamar colorimetric microdilution broth methods for antifungal susceptibility testing of yeast isolates. *J. Clin. Microbiol.* **33**:2660–2664.