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Comparison of a Photometric Method with Standardized Methods of Antifungal Susceptibility Testing of Yeasts

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We determined the fluconazole MICs for 101 clinical isolates of *Candida* and *Cryptococcus neoformans* using the macro- and microdilution methods recommended by the National Committee for Clinical Laboratory Standards. We compared the MICs obtained by these methods with those obtained by a photometric assay that quantified the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) by viable fungi. The MIC determined by this method was defined as the highest fluconazole concentration associated with the first precipitous drop in optical density. For *Candida*, both the MTT and the microdilution methods demonstrated excellent agreement with the standard macrodilution method. The MTT method, however, generated MICs at 24 h that were comparable to those generated by the standard macrodilution method, whereas the microdilution method required 48 h. For *C. neoformans*, the levels of agreement between the MICs determined by the MTT and microdilution methods after 48 h and those determined by the standard 72-h macrodilution method were 94% (29 of 31) and 94% (29 of 31), respectively. The MTT method therefore provided results comparable to those of currently recommended methods and had the advantages of a more rapid turnaround time and potential adaptability to use as an automated system. Furthermore, the MICs determined by the MTT method were determined photometrically, thereby eliminating reader bias.

The methods of antifungal susceptibility testing currently recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (9) have several major shortcomings. The standard method, a broth macrodilution technique, is time-consuming and labor-intensive and requires 48 h for determination of MICs for *Candida*; furthermore, the method is subject to variable interpretation due to trailing endpoints caused by partial inhibition of fungal growth (1, 3, 5, 6, 11). The microdilution technique proposed as an alternative method is easier to perform, but it does not have a shortened turnaround time or eliminate trailing endpoints (1).

Several investigators have incorporated photometric assays into susceptibility testing procedures in attempts to overcome these shortcomings (2, 7, 15, 19). One such assay quantifies mitochondrial respiration by viable fungi through the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to formazan. The fluconazole MICs determined by an MTT method were comparable to those determined by the standard macrodilution method (7). That study, however, included only a few isolates of *Candida albicans*; therefore, the ability of this method to accurately determine the MICs for a large number of *Candida* isolates from different species is unproven.

We hypothesized that an MTT assay might meet the need for a rapid, reliable, and easy-to-perform method of antifungal susceptibility testing that is independent of individual reader bias. We therefore applied an MTT method to determine the MICs of fluconazole for 101 clinical isolates of *Candida* and *Cryptococcus neoformans* and compared the results with those of the broth dilution methods currently recommended by NCCLS.

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MATERIALS AND METHODS

Yeast isolates. Seventy clinical isolates of *Candida* spp. collected during a prospective study of candidemia were tested (10). These included *C. albicans* ($n = 26$ isolates), *Candida tropicalis* ($n = 17$), *Candida glabrata* ($n = 10$), *Candida parapsilosis* ($n = 10$), *Candida krusei* ($n = 4$), and *Candida lusitaniae* ($n = 3$). Thirty-one clinical isolates of *C. neoformans* from human immunodeficiency virus-infected patients obtained from the clinical microbiology laboratory of the Shands Teaching Hospital at the University of Florida (Gainesville, Fla.) or from the Fungus Testing Laboratory at the University of Texas Health Science Center (San Antonio, Tex.) were tested. Isolates were maintained at −70°C and passed at least twice onto Sabouraud dextrose agar (SDA) prior to MIC determination. *C. parapsilosis* ATCC 90018 and *C. neoformans* ATCC 90112 were incorporated into each set of experiments as quality control isolates. Fluconazole MICs for the American Type Culture Collection (ATCC) isolates were determined by all three methods.

Fluconazole. Fluconazole powder was graciously provided by Pfizer Central Research (Groton, Conn.). Stock solutions of 2,000 μg/ml were prepared with sterile distilled water and were stored at room temperature.

Susceptibility testing. Each isolate was tested simultaneously by the macrodilution, microdilution, and MTT methods. Yeast isolates were subcultured onto SDA plates, grown at 35°C for 24 to 48 h, and subcultured again for 24 h prior to testing. Inocula were prepared photometrically by suspending colonies removed from SDA plates in approximately 5 ml of sterile water and adjusting the turbidity of the suspension to match that of a 0.5 McFarland standard.

(i) Macrodilution method. The macrodilution method was performed by the standard method proposed by NCCLS (9).

(ii) Microdilution method. The microdilution method was performed according to the recommendations of NCCLS (9). The fluconazole stock solution was diluted in sterile water by a stepwise dilution scheme to yield concentrations ranging from 0.25 to 64 μg/ml (twice the final test concentrations). Two sterile, flat-bottom, 96-well microtiter plates (Falcon 3072; Becton Dickinson) were used for each group of six isolates tested and were prepared identically. For testing of *Candida* spp., one plate was used for the MTT assay in which the results were read at 24 h, and the other was used for the MTT assay in which the results were read at 48 h. A similar design was applied for testing of *C. neoformans*, but MTT assay results were determined at 48 and 72 h (see below). One hundred microliters of each concentration of fluconazole was dispensed into the microtiter wells, with column 3 of each plate containing the lowest concentration of drug and column 11 containing the highest concentration of drug. The first column of each plate served as a blank, and the second and last columns served as growth controls (i.e., drug-free controls). The top and bottom rows of each plate were blanks containing 200 μl of sterile water. The plates were stored at −70°C and were thawed at room temperature at the time of testing. Working yeast suspen-

TABLE 1. Fluconazole MICs for clinical yeast isolates obtained by macrodilution, microdilution, and MTT methods[a]

Yeast (no. of isolates)	Fluconazole MIC (μg/ml)											
	Macrodilution method				Microdilution method				MTT method			
	Range	Mean	50%	90%	Range	Mean	50%	90%	Range	Mean	50%	90%
C. albicans (26)	0.125–>32	1.2	0.5	>32	0.125–>32	0.9	0.5	8	0.125–>32	1.0	0.5	16
C. tropicalis (17)	0.5–>32	7.3	8	>32	1–>32	6.2	4	>32	0.5–>32	5.5	4	>32
C. glabrata (10)	2–>32	8.5	8	16	2–>32	6.5	8	8	1–>32	7.9	8	16
C. parapsilosis (10)	1–>32	4.0	2	32	1–32	2.8	2	16	1–32	3.2	2	16
C. krusei (4)	≥32	>32	>32	>32	≥32	>32	>32	>32	≥16	>32	>32	>32
C. lusitaniae (3)	0.125–32	2.5	8	32	0.125–>32	2.0	2	>32	0.125–>32	2.0	2	>32
C. neoformans (31)	0.5–32	3.9	4	8	0.5–32	4.4	4	16	0.5–32	4.7	4	16

[a] By the microdilution method, the results were read at 48 h for *Candida* and at 72 h for *C. neoformans*. By the MTT method, the results were read at 24 h for *Candida* and at 48 h for *C. neoformans.*

sions were prepared by diluting yeast inoculum suspensions 1:50 and then 1:20 in RPMI 1640 medium with L-glutamine and without sodium bicarbonate and buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; American Biorganics, Inc., Niagara Falls, N.Y.); 100 μl of working suspension was added to the microtiter wells, yielding final fluconazole concentrations of 0.125 to 32 μg/ml. Since each row (excluding the two blank rows) of the microtiter plate represented a different isolate, it was possible to test up to six isolates on each plate. All plates were incubated at 35°C. MICs were determined at 24 and 48 h for *Candida* and at 48 and 72 h for *C. neoformans*. The MIC was defined as the lowest concentration of fluconazole causing a slightly hazy turbidity compared to the appearance of the growth control well.

(iii) MTT method. After the MICs were visually determined on each of the microtiter plates, the plates were used for MTT testing. Twenty-five microliters of RPMI 1640 medium containing 5 mg of MTT (Sigma Chemical, St. Louis, Mo.) per ml was added to each well containing an inoculum. Incubation was continued at 35°C for 3 h. A stock solution of acid-isopropanol (5 ml of 1 N HCl in 95 ml of isopropanol) was simultaneously warmed for 3 h at 35°C. After 3 h, the plates were centrifuged at 1,000 × *g* for 10 min and the supernatants were aspirated. One hundred microliters of warm acid-isopropanol was added to each well containing an inoculum. The optical density (OD) was determined with a microplate spectrophotometer (Multiskan MCC; Titertek, Santa Barbara, Calif.) at 500 nm and was plotted semilogarithmically against the fluconazole concentration. The MIC was defined as the highest concentration of drug that was associated with the first precipitous drop in OD.

Definitions and terminology. Agreement between methods was defined as a difference in MICs of a fourfold dilution or less (two tubes). Discrepancy referred to a difference of greater than a fourfold dilution.

We used the recently proposed fluconazole breakpoints for *Candida* spp. to classify the isolates as susceptible (fluconazole MIC, ≤8 μg/ml), susceptible-dose dependent (MIC, 16 or 32 μg/ml), or resistant (MIC, ≥64 μg/ml) (13). Data will be given for each of these categories; however, for the purpose of discussion, these categories will be simplified into susceptible or nonsusceptible (susceptible-dose dependent and resistant).

The standard method of susceptibility testing was the 48-h macrodilution method for *Candida* and the 72-h macrodilution method for *C. neoformans*.

Unless specifically stated otherwise, all macrodilution and microdilution results represented MICs for *Candida* read at 48 h and MICs for *C. neoformans* read at 72 h. Unless specifically stated otherwise, all MTT assay results represented MICs read at 24 h for *Candida* and MICs read at 48 h for *C. neoformans*.

RESULTS

Detectable growth was evident by each method for all *Candida* isolates at 24 h and for all *C. neoformans* isolates at 48 h. The fluconazole MICs for the quality control isolates (ATCC 90018 and ATCC 90112) obtained by each method were within the acceptable range (9). For *C. parapsilosis* ATCC 90018, the fluconazole MICs at 24 and 48 h were 0.5 μg/ml by the macrodilution and microdilution methods and the MIC at 24 h was 0.5 μg/ml by the MTT method. For *C. neoformans* ATCC 90112, the fluconazole MICs at 48 and 72 h were 2.0 μg/ml by the macrodilution and microdilution methods and the MIC at 48 h was 2.0 μg/ml by the MTT method.

Testing of *Candida* isolates for susceptibility to fluconazole. There was no significant difference in the ranges of MICs, geometric mean MICs, MICs at which 50% of the isolates are inhibited (MIC_{50}s) or MIC_{90}s for *Candida* spp. by the macrodilution and microdilution methods at 48 h and the MTT method at 24 h (Table 1).

Macrodilution and microdilution methods: agreement between readings at 24 and 48 h. The levels of agreement within twofold and within fourfold between MICs obtained by the macrodilution method at 24 and 48 h were 70% (49 of 70 isolates) and 83% (58 of 70 isolates), respectively (Table 2). Among the 12 isolates for which there were discrepancies in MICs of greater than fourfold, 10 were susceptible to fluconazole at 24 h but were not susceptible at 48 h (Table 3).

Agreement between macrodilution versus microdilution methods. The levels of agreement within twofold and within fourfold between the MICs at 48 h by the microdilution method and the standard 48 h macrodilution method were 90% (63 of 70) and 97% (68 of 70), respectively (Table 2). For the two isolates for which a discrepancy in MICs of greater than fourfold was demonstrated, the MIC determined by the macrodilution method was higher than the MIC determined by the microdilution method. For these isolates, the microdilution MIC was within the range for fluconazole susceptibility, while the macrodilution MIC was outside of the range for susceptibility (Table 3).

The levels of agreement within twofold and within fourfold between the MICs at 24 h by the microdilution method and the MICs at 48 h by the standard macrodilution method were 74% (52 of 70) and 86% (60 of 70), respectively (Table 2). For all 10 isolates for which a discrepancy in MICs of greater than fourfold was demonstrated, the MIC determined at 48 h by the macrodilution method was higher than that determined at 24 h by the microdilution method. For seven of these isolates, the MIC at 24 h by the microdilution method was within the range for fluconazole susceptibility, while the MIC at 48 h by the macrodilution method was outside of the range for susceptibility (Table 3).

Comparison of MTT method with macrodilution and microdilution methods. Representative curves of the fluconazole MICs against the OD are presented in Fig. 1 and 2.

The level of agreement within twofold between the MICs obtained by the MTT method at 24 and 48 h was 61% (43 of 70). The levels of agreement within twofold and within fourfold between the MTT method at 24 h and the macrodilution method at 48 h were 89% (62 of 70) and 94% (66 of 70), respectively (Table 2). For all four isolates for which a discrepancy in MICs between the methods of greater than fourfold was demonstrated, the MIC by the macrodilution method was higher. For three of these isolates, the MIC was within the range for fluconazole susceptibility by the MTT method but outside of the range for susceptibility by the macrodilution method (Table 3). Note that the level of agreement with the

TABLE 2. Agreement in MICs obtained by macrodilution, microdilution, and MTT methods

Discrepancy between methods	% Agreement in MICs															
	Macrodilution method at 24 h/macrodilution method at 48 h			Microdilution method at 24 h/microdilution method at 48 h			Microdilution method at 24 h/macrodilution method at 48 h			MTT method at 24 h/macrodilution method at 48 h			MTT method at 24 h/microdilution method at 48 h		
	C. albicans (26)[a]	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)
≤2-fold	69(18/26)[b]	70(31/44)	70(49/70)	85(22/26)	91(40/44)	89(62/70)	69(18/26)	77(34/44)	74(52/70)	93(24/26)	89(39/44)	90(63/70)	88(23/26)	89(39/44)	89(62/70)	100(26/26)	98(43/44)	99(69/70)
4-fold	8(2/26)	16(7/44)	13(9/70)	4(1/26)	9(4/44)	7(5/70)	12(3/26)	11(5/44)	11(8/70)	4(1/26)	9(4/44)	7(5/70)	4(1/26)	7(3/44)	6(4/70)	0	0	0
8-fold	12(3/26)	2(1/44)	6(4/70)	0	0	0	4(1/26)	9(4/44)	7(5/70)	0	0	0	4(1/26)	0	1(1/70)	0	0	0
16-fold	0	5(2/44)	3(2/70)	4(1/26)	0	1(1/70)	0	0	0	0	2(1/44)	1(1/70)	0	2(1/44)	1(1/70)	0	0	0
32-fold	4(1/26)	5(2/44)	4(3/70)	0	0	0	4(1/26)	2(1/44)	3(2/70)	0	0	0	0	0	0	0	0	0
≥64-fold	8(2/26)	2(1/44)	4(3/70)	8(2/26)	0	3(2/70)	12(3/26)	0	4(3/70)	4(1/26)	0	1(1/70)	4(1/26)	2(1/44)	3(2/70)	0	2(1/44)	1(1/70)

[a] Numbers in parentheses in subheads are numbers of isolates.
[b] Values in parentheses indicate numbers of isolates for which results were in agreement/total numbers of isolates.

standard macrodilution method at 48 h was better for the MTT method at 24 h (94%) than for the microtiter method at 24 h (86%; 60 of 70).

The level of agreement between the MTT method and the microdilution method at 48 h was 99% (69 of 70) (Table 2). The only isolate for which a discrepancy in MIC was exhibited was a *C. tropicalis* isolate that was susceptible to fluconazole by the MTT method (MIC, 1.0 μg/ml) but that was not susceptible by the microdilution method (MIC, >32 μg/ml).

Testing of *C. neoformans* isolates for susceptibility to fluconazole. There was no significant difference between the three methods in the ranges of MICs, geometric mean MICs, MIC_{50}s, or MIC_{90}s for the isolates of *C. neoformans* (Table 1).

The levels of agreement of MICs within twofold and within fourfold were as follows: macrodilution method at 48 and 72 h, 93% (29 of 31) and 97% (30 of 31), respectively; microdilution method at 48 and 72 h, 97% (30 of 31) and 100% (31 of 31), respectively; microdilution method at 48 h and macrodilution method at 72 h, 100% (31 of 31); microdilution method at 72 h and macrodilution method at 72 h, (30 of 31), respectively; and MTT method at 48 h and macrodilution method at 72 h, 93% (29 of 31) and 100% (31 of 31), respectively.

DISCUSSION

We demonstrated excellent agreement between the results of a photometric method of fluconazole susceptibility testing that quantifies the reduction of MTT and those obtained by the recommended standard broth macrodilution method for 101 clinical yeast isolates. The levels of agreement within twofold and within fourfold between MICs obtained by the MTT method and standard macrodilution methods were 89% (62 of 70) and 94% (66 of 70), respectively, for *Candida* spp. and 93% (29 of 31) and 100% (31 of 31), respectively, for *C. neoformans* isolates. Our results confirm those of an earlier study which found that fluconazole MICs determined by an MTT method were comparable to those determined by the reference macrodilution method (7). The previous study, however, included only a few isolates of *C. albicans*. Our study is therefore the first to demonstrate the ability of an MTT assay to accurately determine the MICs for a large number of *Candida* isolates of different species, as well as for *C. neoformans*. The excellent agreement between the MTT method and the standard macrodilution method shown in our study suggests that the MTT method might be a viable alternative to the current standard method for antifungal susceptibility testing.

A previous study found that the addition of menadione, which augments the reduction of MTT to formazan, was necessary to increase the sensitivity of the assay (7). We found this step to be unnecessary; in fact, the addition of menadione in our preliminary studies resulted in less consistent endpoint determinations, higher MICs, and lower levels of agreement with the reference method. Furthermore, we found that the extraction of formazan, which can be cumbersome and time-consuming (8, 14), was not a problem if acid-isopropanol was warmed to 35°C prior to its addition to the microtiter wells.

To date, a microdilution adaptation of the macrodilution method is the only approved alternative method of antifungal susceptibility testing (9). Our study confirms previous findings that the MICs obtained by the microdilution method agree well with those obtained by the standard macrodilution method (1): we obtained levels of agreement of 97% (68 of 70) for *Candida* and 100% (31 of 31) for *C. neoformans* between these two methods. Our study also confirms previous findings that the microdilution method requires 48 h to yield results comparable to those of the standard macrodilution method

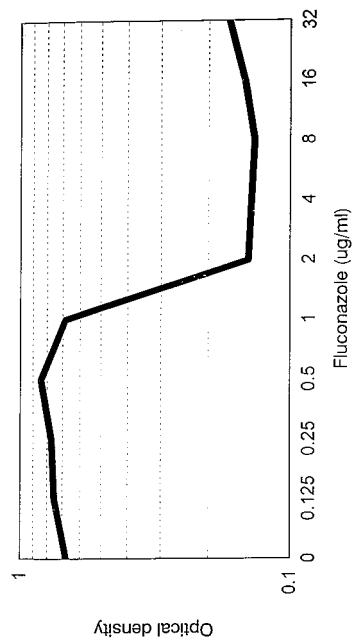

FIG. 1. Representative curve generated by the MTT method for isolates of *C. albicans*. The MIC was defined as the highest concentration of fluconazole that was associated with the first precipitous drop in OD; for this isolate, the MIC was 2 μg/ml.

(1). Indeed, for *Candida*, the levels of agreement within twofold and within fourfold between the microdilution method at 24 h and the standard macrodilution method at 48 h were only 74% (52 of 70) and 86% (60 of 70), respectively; the levels of agreement improved to 90% (63 of 70) and 97% (68 of 70), respectively, when the reading time was extended to 48 h. Importantly, among the 10 *Candida* isolates for which there was a greater than fourfold discrepancy in MICs between the microdilution method at 24 h and the standard macrodilution method at 48 h, 7 demonstrated differences in susceptibility interpretation patterns: they were all considered susceptible to fluconazole by the former method but not susceptible by the latter method.

The MTT method was superior to the microdilution method in that it required only 24 h to demonstrate good agreement with the results of the standard macrodilution method at 48 h for *Candida*: the levels of agreement within twofold and within fourfold were 89% (62 of 70) and 94% (66 of 70), respectively. These levels of agreement were superior to those between the microdilution method at 24 h and the standard macrodilution method at 48 h and were comparable to those between the microdilution method at 48 h and the macrodilution method at 48 h (Table 2). Furthermore, in contrast to the 10% (7 of 70) difference in susceptibility interpretation pattern seen between the microdilution method at 24 h and the standard macrodilution method at 48 h, only 4% (3 of 70) of the isolates demonstrated a difference in susceptibility interpretation pattern

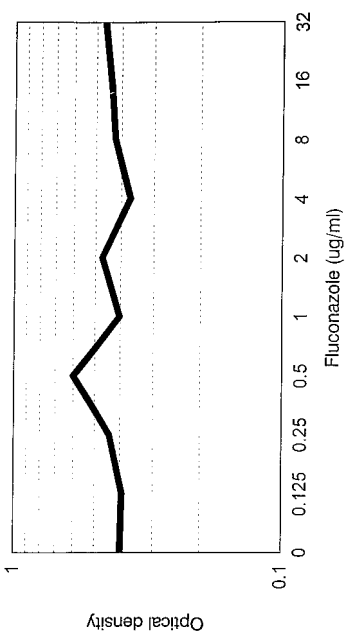

FIG. 2. Representative dose-response curve generated by the MTT method for an isolate of *C. tropicalis*. There was no drop in OD; the MIC was therefore >32 μg/ml.

TABLE 3. Discrepancy in MICs obtained by macrodilution, microdilution, and MTT methods causing changes in susceptibility interpretative pattern

Discrepancy[a]	% Agreement in MICs																	
	Macrodilution method at 24 h/macrodilution method at 48 h			Microdilution method at 24 h/microdilution method at 48 h			Microdilution method at 24 h/macrodilution method at 48 h			Microdilution method at 48 h/macrodilution method at 48 h			MTT method at 24 h/macrodilution method at 48 h			MTT method at 24 h/ microdilution method at 48 h		
	C. albicans (26)[b]	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)	*C. albicans* (26)	Non-*C. albicans* (44)	Total (70)
S/S	8(2/26)[c]	0	3(2/70)	4(1/26)	0	1(1/70)	4(1/26)	5(2/44)	4(3/70)	0	0	0	4(1/26)	0	1(1/70)	0	0	0
S/S-DD	4(1/26)	9(4/44)	7(5/70)	0	0	0	4(1/26)	7(3/44)	6(4/70)	0	1(1/70)	1(1/70)	0	2(1/44)	1(1/70)	0	0	0
S/R	12(3/26)	5(2/44)	7(5/70)	8(2/26)	0	3(2/70)	12(3/26)	0	4(3/70)	4(1/20)	0	1(1/70)	4(1/26)	2(1/44)	3(2/70)	0	2(1/44)	1(1/70)

[a] Discrepancy, difference in MICs of greater than fourfold between methods; S/S discrepancy, MICs by both methods within susceptible range; S/S-DD discrepancy, MIC in susceptible range by one method and in susceptible-dose dependent range by other method; S/R discrepancy, MIC in susceptible range by one method and in resistant range by other method (susceptible, MIC ≤8 μg/ml; susceptible-dose dependent, MIC 16 to 32 μg/ml; resistant, MIC ≥ 64 μg/ml). For the purpose of discussion in the text, susceptible-dose dependent and resistant are referred to as nonsusceptible.

[b] See footnote *a* to Table 2.

[c] See footnote *b* to Table 2.

between the MTT method at 24 h and the standard macrodilution method at 48 h (Table 3).

In addition to the ease of testing and the short turnaround time, the MTT method yielded results that were easy to interpret. The spectrophotometric determination of MICs is more objective than the visual assessment required for the macro- and microdilution methods. This is a particular advantage of the MTT assay, given the well-described phenomenon of trailing endpoints associated with in vitro testing of the susceptibilities of yeasts to azole agents.

In addition to the MTT assays, a variety of other methods have been studied as potential alternatives to the standard macrodilution method. The most commonly studied method uses the incorporation of a commercially available oxidation-reduction indicator (Alamar Blue; Alamar Biosciences Inc., Sacramento, Calif.) into the microdilution method (4, 12, 16–18). Overall, the level of agreement between this method and the standard method was good against a wide range of clinical yeast isolates. Furthermore, like the MTT assay, it yielded accurate results at 24 h (16, 17). There are relative advantages and disadvantages to each method, however. The Alamar Blue method is easier to perform than the MTT method, since it does not require the addition of electron-coupling agents or centrifugation. On the other hand, unlike the MTT method, the Alamar Blue method still relies on the subjective interpretation of slight color changes to define the MICs.

Spectrophotometric assessment rather than visual assessment of MIC endpoints has also been studied as an alternative method of susceptibility testing. Excellent agreement with the results of the standard method was demonstrated (2, 4). Reading of the results of the spectrophotometric method at 24 h has been shown to be accurate and is simpler to perform than the colorimetric assays.

At this point, however, experience with any of the alternative colorimetric or spectrophotometric methods is too limited to assess the relative superiority of any method. Further investigation of all these methods, including studies directly comparing methods, is warranted. Particularly important will be assessment of the abilities of different methods to generate MICs that correlate with the in vivo response to therapy.

In conclusion, we have shown that a photometric method of susceptibility testing by an MTT assay not only provided good agreement with the standard macrodilution method but did so in 24 h less time. The advantages of the MTT method include the simplicity of the procedure, the rapid turnaround time, the easily interpretable endpoint determinations, and the potential adaptability to use as an automated system. The results of our study suggest that the MTT method should be included in future investigations of alternatives to the current standard macrodilution method.

REFERENCES

1. **Barchiesi, F., A. I. Colombo, D. A. McGough, and M. G. Rinaldi.** 1994. Comparative study of broth macrodilution and microdilution techniques for in vitro antifungal susceptibility testing of yeasts by using the National Committee for Clinical Laboratory Standards' proposed standard. J. Clin. Microbiol. **32:**2494–2500.
2. **Barchiesi, F., M. Del Poeta, V. Morbiducci, F. Ancarani, and G. Scalise.** 1993. Turbidimetric and visual criteria for determining the in vitro activity of six antifungal agents against *Candida* spp. and *Cryptococcus neoformans*. Mycopathologia **124:**19–25.
3. **Espinel-Ingroff, A., C. W. Kish, T. M. Kerkering, R. A. Fromtling, K. Bartizal, J. N. Galgiani, K. Villareal, M. A. 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.
4. **Espinel-Ingroff, A., J. L. Rodriguez-Tudela, and J. V. Martinez-Suarez.** 1995. Comparison of two alternative microdilution procedures with the National Committee for Clinical Laboratory Standards reference macrodilution method M27-P for in vitro testing of fluconazole-resistant and -susceptible isolates of *Candida albicans*. J. Clin. Microbiol. **33:**3154–3158.
5. **Espinel-Ingroff, A., T. M. Kerkering, P. R. Goldson, and S. Shadomy.** 1991. Comparison study of broth macrodilution and microdilution antifungal susceptibility tests. J. Clin. Microbiol. **29:**1089–1094.
6. **Fromtling, R. A., J. N. Galgiani, M. A. Pfaller, A. Espinel-Ingroff, K. F. Bartizal, M. S. Bartlett, B. A. Body, C. Frey, G. Hall, G. D. Roberts, F. B. Nolte, F. C. Odds, M. G. Rinaldi, A. M. Sugar, and K. Villareal.** 1993. Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. Antimicrob. Agents Chemother. **37:**39–45.
7. **Jahn, B., E. Martin, A. Stueben, and S. Bhakdi.** 1995. Susceptibility testing of *Candida albicans* and *Aspergillus* species by a simple microtiter menadione-augmented 3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. J. Clin. Microb. **33:**661–667.
8. **Meshulam T., S. M. Levitz, L. Christin, and R. D. Diamond.** 1995. A simplified new assay for assessment of fungal cell damage with the tetrazolium dye, (2,3)-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT). J. Infect. Dis. **172:**1153–1156.
9. **National Committee for Clinical Laboratory Standards.** 1995. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Nguyen, M. H., J. E. Peacock, Jr., A. J. Morris, et al.** 1996. The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. Am. J. Med. **100:**617–623.
11. **Pfaller, M. A., and M. G. Rinaldi.** 1993. Antifungal susceptibility testing: current status of technology, limitations, and standardization. Infect. Dis. Clin. N. Am. **7:**435–444.
12. **Pfaller, M. A., and A. L. Berry.** 1994. Evaluation of a novel colorimetric broth microdilution method for antifungal susceptibility testing of yeast isolates. J. Clin. Microbiol. **32:**1992–1996.
13. **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.
14. **Scudiero, D. A., R. H. Shoemaker, K. D. Paull, et al.** 1988. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res. **48:**4827–4833.
15. **Tellier, R., M. Krajden, G. A. Grigoriew, and I. Campbell.** 1992. Innovative endpoint determination system for antifungal susceptibility testing of yeasts. Antimicrob. Agents Chemother. **36:**1619–1625.
16. **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.
17. **Tiballi, R. N., L. T. Zarins, X. He, and C. A. Kauffman.** 1995. *Torulopsis glabrata*: Azole susceptibilities by microdilution colorimetric and macrodilution broth assays. J. Clin. Microbiol. **33:**2612–2615.
18. **To, W., 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.
19. **Van der Auwera, P., A. M. Ceuppens, C. Heymans, and F. Meunier.** 1986. In vitro evaluation of various antifungal agents alone and in combination by using an automatic turbidimetric system combined with viable count determinations. Antimicrob. Agents Chemother. **29:**997–1004.