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Microdilution Antifungal Susceptibility Testing of *Candida albicans* and *Cryptococcus neoformans* with and without Agitation: an Eight-Center Collaborative Study

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The growth patterns observed in the trailing wells when fluconazole is being tested may give rise to readings that suggest resistance or increased MICs for known susceptible strains. We conducted a multicenter study to evaluate the intralaboratory and interlaboratory reproducibilities of a method that uses agitation to disperse these types of growth. Ten strains of *Candida albicans* and five strains of *Cryptococcus neoformans* were tested against fluconazole, flucytosine, and amphotericin B by using a microdilution adaptation of the proposed reference method of the National Committee for Clinical Laboratory Standards for yeasts (M27-T). The endpoint criterion used before agitation was consistent with the M27-T recommendation, while a criterion of 50% or more reduction of growth compared with the control was used after agitation. The results of this study showed that use of agitation and the modified endpoint criterion both improved intralaboratory and interlaboratory agreement and increased the frequency of interpretable MICs. The MICs obtained by this method were comparable to those obtained by the broth macrodilution M27-T method. Like M27-T, this method was not able to definitely distinguish amphotericin B-susceptible from -resistant strains, although the MICs for the resistant strains were consistently higher than those for the susceptible ones. The findings imply that agitation should be seriously considered when antifungal agents, particularly fluconazole, are tested in a microdilution format.

Although significant progress towards development of standardized susceptibility testing methods for antifungal agents has been achieved through the continuing efforts of the National Committee for Clinical Laboratory Standards and others (4, 6, 7, 9, 10, 12–15, 18), the proposed broth macrodilution method is cumbersome and is not practical for use in routine laboratory settings. We have recently reported a simpler broth microdilution method, with agitation of the plates before reading, that improved detection of MIC endpoints (1). An important feature of this new method is its effect on trailing endpoints. The trailing effect is especially prominent when fluconazole is tested and ranges from well-defined buttons with easily discernible circumferences but not too easily recognizable thicknesses to a thin layer of growth entirely coating the walls of the wells in the microdilution plate. These growth patterns may give rise to readings that suggest resistance or increased MICs for known susceptible strains. In our previous study, agitation dispersed both these types of growth and, when used in conjunction with a recommended endpoint criterion, simplified MIC determination. It also enhanced the correlation between in vitro results and in vivo outcome (1, 2). To further evaluate this method, we conducted a multicenter study evaluating the intralaboratory and interlaboratory reproducibility of this method. The eight participating centers used this method to test the susceptibility of 10 strains of *Candida albicans* and of 5 strains of *Cryptococcus neoformans* to fluconazole, flucytosine, and amphotericin B.

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MATERIALS AND METHODS

Antifungal agents. Fluconazole powder (Pfizer Central, Sandwich, United Kingdom), flucytosine (Roche Laboratories, Nutley, N.J.), and amphotericin B (E. R. Squibb and Sons, Princeton, N.J.) from single lots were used.

Test organisms. Ten strains of *Candida albicans*, including nine American Type Culture Collection (ATCC) strains (ATCC 64544 to 64547 and ATCC 64549 to 64553) and R89-335, an isolate kindly provided by M. Rinaldi of the Fungus Testing Laboratory, San Antonio, Tex., were tested. Five isolates of *Cryptococcus neoformans*, R87-29, R87-108, R90-51 (all supplied by M. Rinaldi), B-3501 (National Institutes of Health), and LJM (University of Texas M. D. Anderson Cancer Center), were also tested. Three differently coded sets of the same isolates were sent to each of the participating laboratories. Isolates labeled fluconazole control (ATCC 64545), flucytosine control (ATCC 64551), and amphotericin B control (ATCC 64552) were also included. This group of isolates includes strains ATCC 64550 and R89-335, for which the MICs of fluconazole are known to be higher than those for other organisms (1, 2); ATCC 64545 and 64553, which are known to be resistant to flucytosine in vivo and for which the MICs of flucytosine in vitro are known to be higher than those for other organ-

isms (2, 10); and ATCC 64552, for which the MIC of amphotericin B (10) is known to be higher than those for the other organisms. Although the in vivo consequences of these higher MICs have not been fully assessed, these strains were included to provide a gauge of how well the agitation method is able to distinguish widely disparate MICs for *C. albicans*. R87-108, a strain of *C. neoformans* for which the MIC of flucytosine is known to be higher than that for the other organisms, was included to distinguish widely disparate MICs for *C. neoformans.*

Assay medium. A single lot of RPMI 1640 medium powder with L-glutamine and morpholinepropanesulfonic acid (MOPS) buffer at 34.53 g/liter and pH 7.0 (American Biorganics, Inc., North Tonawanda, N.Y.) was prepared by following the manufacturer's directions. The lot was filter sterilized by using 0.22-nm-pore-size filters, aliquoted, and stored at 4°C until use.

Drug dilution. Twofold drug dilutions in RPMI 1640 medium were prepared from stock solutions of antifungal agents as previously described (6). The stock solution concentrations of fluconazole and flucytosine were 32,000 μg/ml in dimethyl sulfoxide, and the test concentrations ranged from 64 to 0.125 μg/ml. The stock solution concentration of amphotericin B was 16,000 μg/ml in dimethyl sulfoxide and test concentrations ranged from 16.0 to 0.03125 μg/ml.

Inoculum preparation. Yeast inocula were prepared as previously described (13). Briefly, yeasts were grown on Sabouraud dextrose agar for 24 h (*C. albicans*) or for 48 h (*C. neoformans*). The inoculum suspension was prepared by picking five colonies at least 1 mm in diameter and suspending the material in 5 ml of 0.85% saline. The turbidity of the suspension was adjusted with a spectrophotometer to 75% transmittance at 530 nm. The saline suspension was diluted 1:50 (0.1 ml plus 4.9 ml) with RPMI 1640 medium to yield a final inoculum concentration of approximately 0.2×10^5 to 1×10^5 CFU/ml. Microdilution wells were inoculated with 0.1 ml of the resulting suspension. The final inoculum concentration after dilution with 0.1 ml of the drug solutions was 1×10^4 to 5×10^4 CFU/ml. The inoculated plates were then incubated at 35°C for 24 to 48 h.

Time of reading. MIC readings were performed after 24 h for *C. albicans* and after 48 h for *C. neoformans.*

Endpoint criteria. Growth in each well was estimated visually and then scored as follows: 0, optically clear; 1+, slightly hazy, i.e., turbidity of more than 0 to 25% of that of the drug-free growth control; 2+, turbidity of more than 25 to 50% of that of the growth control; 3+, turbidity of more than 50 to 75% of that of the growth control; and 4+, turbidity of more than 75 to 100% of that of the growth control (13). The MIC endpoint before agitation for all drugs was the lowest drug concentration giving a score of ≤1+. In addition, a separate before-agitation (BA) MIC endpoint for amphotericin B was determined, following the National Committee for Clinical Laboratory Standards' M27-T guidelines, as the lowest drug concentration giving a score of 0. For the after-agitation (AA) readings, the MIC endpoint for all drugs was the lowest drug concentration producing at least a 50% reduction in growth or a change in score of at least 2+ (1). MIC readings were performed BA and AA with a two-plate microdilution plate shaker (Dynatech Laboratories, Inc., Alexandria, Va.). The agitation was considered adequate when the growth control well showed homogeneity, usually achieved at a calibration setting of 50 for 5 min (1).

Study design and analysis of results. Eight laboratories (centers A through H) participated in the study. Each laboratory received three complete sets of all study isolates, each coded differently, which were assessed by both test methods on each of the three successive days of testing. Results were recorded on data sheets supplied to each of the laboratories and submitted to the coordinating laboratory upon completion.

Statistical analyses. By study design, there should be a total of three BA and AA paired readings for each organism at each center. Paired BA and AA MICs that fell within 2 twofold dilutions were considered in agreement. MICs that were higher than the highest concentration tested (i.e., >64 μg/ml) were considered arbitrarily uninterpretable and were assigned a value of 128 μg/ml for the purpose of analysis. If three or more replicate analyses of a given organism were performed, intralaboratory agreement was assessed by counting the number of replicates per organism for which the MICs fell within 2 twofold dilutions of the median value for that organism for that center (12). If only two MICs were available, they were counted as being in agreement if they fell within 2 twofold dilutions of each other. Interlaboratory agreement was determined by comparing the overall median MIC obtained for a particular isolate in determinations at all centers with the median MIC obtained by each of the participating laboratories for that isolate. One measure of the contribution of agitation is whether this technique increases the overall number of interpretable readings in the putatively fluconazole-susceptible (fluconazole group A) and fluconazole-resistant (fluconazole group B) strains. While no officially recognized breakpoints yet exist, for the purpose of this study fluconazole-susceptible isolates were defined as those for which the M27-T MIC was ≤4 μg/ml, whereas fluconazole-resistant isolates were those for which the M27-T MIC was ≥8 μg/ml. This determination was made among complete pairs, since it is likely that the BA and AA results for a particular replicate analysis for a given organism and laboratory are correlated. The odds ratio was used to examine the proportions of interpretable and in-agreement results BA and AA. The McNemar test was used to examine the gain in interpretability after agitation. Kruskal-Wallis and χ^2 tests were used to compare the distribution of MICs. All tests of significance were two sided and considered significant at the 0.05 level. Analyses were done separately for fluconazole groups A and B. A second analysis, which made no distinction for the pairing of BA and AA readings, was also undertaken.

Spectrophotometric evaluation. No spectrophotometric readings were required in the protocol, but two centers voluntarily performed this evaluation procedure on a limited number of strains and recorded the method used. One center (center H) used a microdilution plate scanner set at 525 nm, while the other (center A) used a similar scanner set at 570 nm. Plates were agitated as prescribed in the protocol. Briefly, the MIC endpoint was considered achieved in the wells that recorded a 50% or more reduction in the optical density reading compared with the optical density reading of the growth control well.

Comparison with M27-T. The 10 strains of *C. albicans* were also tested by the M27-T reference macrodilution and microdilution test methods (12). The M27-T microdilution method results are given as the MIC 1+ BA results. The median MICs obtained in this study BA and AA were compared with those MICs obtained by the testing center, which used procedures without modifications, i.e., as specified in the M27-T protocol.

RESULTS

Protocol compliance. Examination of the data revealed good compliance with the protocol by all centers except two, one that provided a much lower number of records than expected and another that performed a much higher number of replicates than expected for all the drugs tested. Analyses were inclusive of all data, including replicates in excess of what the protocol specified, unless stated otherwise.

Intralaboratory and interlaboratory agreement for *C. albicans*. (i) Fluconazole. The overall intralaboratory agreement for fluconazole group A ranged from 61 to 100% for MICs determined BA (average, 92%) and from 75 to 100% for MICs determined AA (average, 87%). The overall intralaboratory reproducibility for fluconazole group B was 96% for MICs obtained BA and 98% for MICs obtained AA. The intralaboratory reproducibility results for the combined fluconazole groups were comparable: 94% for MICs obtained BA and 92% for MICs obtained AA.

Interlaboratory agreement was also good. Overall, 77% of the BA readings (median MIC, 128 μg/ml) and 62% of the AA readings (median MIC, 0.5 μg/ml) varied fourfold or less among the eight centers. However, when the uninterpretable results (MIC, >64 μg/ml) were excluded, the overall agreement AA increased to 79% (median MIC, 0.5 μg/ml). More importantly, for 84% of the strains for which the BA MIC was ≥8 μg/ml, the AA MIC was ≤4 μg/ml. Of the 224 BA determinations, 201 (90%) were uninterpretable (>64 μg/ml). For the 10% (23 of 224) that were interpretable, the median MIC was 16 μg/ml. Data obtained earlier by visual and spectrophotometric in vitro testing and by in vivo studies had shown these strains to be susceptible to fluconazole (1, 2).

When all data were considered, the median BA MIC for all the isolates tested was >64 μg/ml (90% of the MICs exceeded 64 μg/ml) and the median AA MIC was 0.5 μg/ml (range, 0.25 to 16 μg/ml). When only interpretable data were considered, the ranges and medians were 0.5 to 32 μg/ml and 16 μg/ml, respectively, for the BA MICs and 0.25 to 16 μg/ml and 0.5 μg/ml, respectively, for the AA MICs. On the basis of the analysis of paired BA and AA MICs, the increase in the number of interpretable MICs resulting from agitation for fluconazole groups A and B across centers was statistically significant (Table 1). In addition, with agreement defined as an MIC within 1 twofold dilution of the median MIC of the interpretable readings for a particular set of data, the odds of agreement in each case were approximately four times higher for the AA results (Table 1). Figure 1 compares the median MIC obtained for each of the strains by the different centers at each of the endpoints used in the study with the reference M27-T macrodilution MIC. This figure illustrates the significant difference between the abilities of the two endpoints to distinguish the putatively susceptible strains (fluconazole group A;

TABLE 1. Improvement in interpretability and agreement of fluconazole MICs by agitation

Fluconazole group and likelihood	Likelihood expressed as:	
	OR (95% CI)[a]	P^b
A		
Likelihood of an interpretable MIC[c]	3.6 (2.2–5.9)	<0.001
Likelihood of an interpretable MIC in agreement[d]	3.0 (1.9–4.5)	<0.001
B		
Likelihood of an interpretable MIC	4.3 (1.5–12.4)	0.01
Likelihood of an interpretable MIC in agreement	4.1 (1.5–10.8)	0.01

[a] Odds ratio (OR) (95% confidence interval [CI]) for the likelihood that the fluconazole MIC obtained AA was an improvement over the MIC obtained BA.
[b] *P* value by the McNemar test.
[c] MIC that is ≤64 μg/ml.
[d] MIC that is within 1 twofold dilution of the median MIC.

M27-T MIC, ≤4 μg/ml) from the resistant strains (fluconazole group G; MIC, ≥8 μg/ml). The median of the MICs obtained AA by all centers was within 1 dilution of the M27-T MIC for 7 of the 10 strains and differed by only 2 dilutions in strains ATCC 64545, ATCC 64546, and ATCC 64552. Strains ATCC 64545 through ATCC 64549 yielded higher MICs in one or more centers even when the MICs were read AA, although only two centers (B and F) obtained AA readings that were grossly disparate from the M27-T MIC. Importantly, all centers obtained comparable MICs for the two resistant strains.

(ii) Flucytosine. The intralaboratory reproducibility for flucytosine MICs was 90 to 100% BA and 93 to 100% AA, with averages of 97 and 99%, respectively. The interlaboratory reproducibility ranged from 79 to 100% for MICs obtained BA and from 88 to 100% for MICs obtained AA. The respective overall averages were 92 and 96%. The MIC ranges and median MICs were 0.125 to 128 μg/ml and 1 μg/ml, respectively, BA and 0.125 to 128 μg/ml and 0.125 μg/ml, respectively, AA. MICs obtained AA were more consistent than those obtained BA, and the median MICs were the same or lower (one well lower for four strains, two wells lower for two strains, and no difference for the remaining four strains). Both endpoint criteria were consistently able to separate the two strains for which the MIC was >64 μg/ml from the eight isolates for which the MIC was ≤2 μg/ml.

(iii) Amphotericin B. The intralaboratory agreements for amphotericin B at endpoints of 0 BA, 1+ BA, and 2+ AA were 90, 99.1, and 99.5%, respectively.

The interlaboratory reproducibility ranged from 43 to 100% at an endpoint of 0 BA, 50 to 100% at 1+ BA, and 38 to 100% at 2+ AA. The respective averages were 84, 87, and 86%. The MICs were generally much higher for centers A and B than for the remaining centers. The MIC ranges and median MICs were 0.25 to 2 μg/ml and 0.25 μg/ml, respectively, for an endpoint of 0 BA, 0.125 to 1.0 μg/ml and 0.125 μg/ml, respectively, for an endpoint of 1+ BA, and 0.0625 to 0.5 μg/ml and 0.125 μg/ml, respectively, for an endpoint of 2+ AA. All centers were able to detect a higher MIC for the test isolate (ATCC 64552) for which the value was known to be higher by using endpoints of 2+ AA and 1+ BA but not by using 0 BA.

Intralaboratory and interlaboratory agreements for *C. neoformans*. (i) Fluconazole. The intralaboratory agreements of fluconazole MICs for *C. neoformans* were 95.6% BA (median MIC, 8 μg/ml) and 90% AA (median MIC, 2 μg/ml). The interlaboratory agreements of fluconazole MICs were 72% BA and 79% AA. The MICs obtained AA by all centers were either the same as or lower than those obtained BA (within ≤2 wells in five of eight centers and >2 wells in the remaining three). One center reported MICs >2 wells higher for all organisms tested than those of the other centers for all endpoint criteria. When all BA and AA MICs were analyzed by the Kruskal-Wallis test, it was found that the differences in median MICs were statistically significant ($P < 0.001$).

(ii) Flucytosine. The intralaboratory agreements of flucytosine MICs were 92% BA and 95% AA. The interlaboratory agreements were 85% BA (median MIC, 8 μg/ml) and 80% AA (median MIC, 2 μg/ml). With the exception of one strain at one center, AA MICs obtained by all centers were the same as or lower than BA MICs (80% differed by ≤2 wells; 14% differed by more than 2 wells). Using MICs obtained AA, all centers but one were able to detect the strain for which the MIC was known to be high. Using MICs obtained BA, all but one center detected the strain for which the MIC was high; the remaining center reported high values for three organisms for which lower values were expected. When all MICs obtained BA were compared with those obtained AA by the Kruskal-Wallis test, the difference in the median MICs was statistically significant ($P < 0.001$).

(iii) Amphotericin B. The intralaboratory agreements of the MICs were 95.8% (endpoint, 0 BA), 99.2% (endpoint, 1+ BA), and 100% (endpoint, 2+ AA). The interlaboratory agreements were 74.8% (median MIC, 0.5 μg/ml) at an endpoint of 0 BA, 91.6% (median MIC, 0.25 μg/ml) at an endpoint of 1+ BA, and 84% (median MIC, 0.125 μg/ml) at an endpoint of 2+ AA. Two centers obtained higher MICs for all isolates than all other centers. Twenty percent (8 of 40) of the MICs obtained at an endpoint of 0 BA were >2 wells higher ("resistant values") than those obtained at an endpoint of 1+ BA or at an endpoint of 2+ AA. MICs obtained at an endpoint of 1+ BA were comparable to those obtained at an endpoint of 2+ AA (97% were within 2 twofold dilutions of each other). The Kruskal-Wallis test was used to examine the significance of the differences in median MICs at each of the different endpoints. By comparing 0+ BA to 1+ BA, 0+ BA to 2+ AA, and 1+ BA to 2+ AA, it was found that the differences were statistically significant ($P = 0.001$, $P < 0.001$, and $P = 0.021$, respectively). When adjusted for ties, the first two probabilities remained the same, whereas the third became 0.019.

DISCUSSION

We have previously shown that agitation in conjunction with a modified inoculum and endpoint criterion simplified MIC determinations by comparison with the M27-T reference macrodilution method. This modified method also enhanced the correlation between in vitro results and in vivo outcome (1, 2). In this study, we have shown that this method has good to excellent reproducibility in a multilaboratory comparison. Data from two centers showed much higher MICs of amphotericin B across all strains, which could be attributable to differences in drug dilution. The reason the MICs of flucytosine obtained by another center for both *C. neoformans* and *C. albicans* were higher than those obtained by the rest of the centers could not be pinpointed. The increased total volumes used in this study may have contributed to a loss of agitation effectiveness and consequently may have led to the higher MICs obtained by some centers. Also, the inoculum size used in this study is 10-fold greater than the current M27-T recommendation, and this too may have contributed to higher MICs obtained by some of the centers, because increased growth

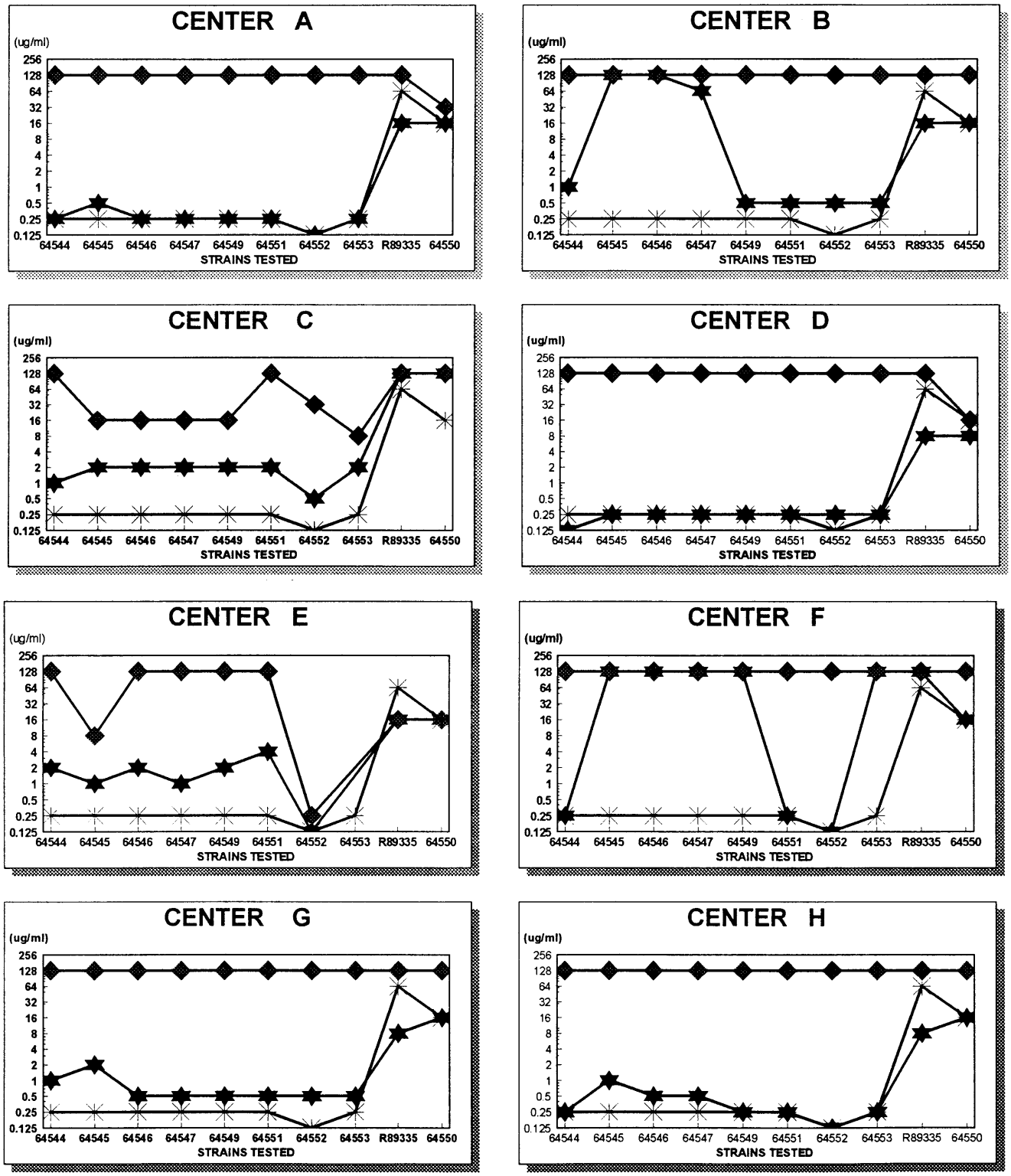

FIG. 1. Fluconazole MICs for *C. albicans*. Shown are the median fluconazole MICs obtained at each of the eight study centers for each of the 10 isolates of *C. albicans*, as determined by reading in a modified microdilution format with a turbidity endpoint of 1+ BA (◆) or 2+ AA (✶). Also shown are the MICs obtained by the M27-T macrodilution method (✳).

observed in the trailing wells may have posed difficulty for some observers. Despite these limitations, the percent intralaboratory agreement remained high for all aspects of comparison without regard to drug, organism, or endpoint criterion. This further supports the finding that variation in in vitro laboratory results cannot usually be attributed to intralaboratory errors (4, 5, 10, 14, 15).

Improved methods of in vitro testing for azole antifungal

agents have used different testing formats (broth macrodilution, broth microdilution semisolid agar microdilution, disk diffusion), different endpoint criteria (IC-1/2, spectrophotometric criteria, visual growth estimation, zone diameters, colorimetric criteria), and various additives (inhibitors, such as detergents and antibiotics, and stimulants, such as increased sugars, vitamins, and other medium supplements) (18, 19). The improvement in the frequency of interpretable MICs in this study came as a result of both the use of a less stringent requirement for growth reduction and mechanical agitation. The effect of the endpoint requirement is in agreement with findings of Fromtling et al. (8), who observed that the modal distributions for ketoconazole MICs were uniformly higher, particularly for *C. albicans*, at a turbidity endpoint of 0 than at an endpoint of 1+ or 2+. The effect of agitation was at times quite striking, and especially so for fluconazole. While agitation does not eliminate trailing, it does render it interpretable for most observers. Obtaining reproducible results for azole antifungal agents has long been difficult, and this simple modification clearly enhances agreement. Although the contribution of agitation to the flucytosine and amphotericin B results was not as dramatic as its contribution to those for fluconazole, agitation did improve the consistency of the results. Our results were also consistent with the findings of Espinel-Ingroff et al. (7), who showed a better agreement with the use of a 2+ endpoint without agitation. Other recent studies that employed agitation with the less stringent endpoint (2+) have also shown similar findings (3, 11, 16, 18).

A comparative analysis of the M27-T and broth microdilution protocols reveals more similarities than differences. Our method uses the same medium, buffer, incubation temperature, and inoculum preparation as the M27-T method. Our method differs from the M27-T method in the volumes used, the endpoint criterion for fluconazole (>50% reduction versus 80% reduction for M27-T), and the incubation time (24 versus 48 h). Our results were comparable to those obtained by the M27-T method (Fig. 1) and to those obtained by others (3, 7, 16, 18). Like the M27-T method (17), our method was not able to definitely distinguish putatively amphotericin B-susceptible from -resistant strains. Interestingly, the MICs for the resistant strain were somewhat higher than those for the susceptible ones if a 2+ AA or 1+ BA endpoint was used but not if the M27-T-recommended 0 BA endpoint was used. However, the difference between the MICs of putatively susceptible and resistant organisms remains narrow, and the interpretation of amphotericin B MICs requires caution and is an area under active development. Agitation and the use of less stringent endpoint criteria should be considered when antifungal agents are tested in a microdilution format, particularly fluconazole.

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REFERENCES

1. **Anaissie, E., V. Paetznick, and G. P. Bodey.** 1991. Fluconazole susceptibility testing of *Candida albicans*: microtiter method that is independent of inoculum size, temperature, and time of reading. Antimicrob. Agents Chemother. **35:**1641–1646. (Erratum, **36:**1170, 1992.)
2. **Anaissie, E. J., N. C. Karyotakis, R. Hachem, M. C. Dignani, J. H. Rex, and V. Paetznick.** 1994. Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. J. Infect. Dis. **170:**384–389.
3. **Barchiesi, F., A. L. 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.
4. **Calhoun, D. L., G. D. Roberts, J. N. Galgiani, J. E. Bennett, D. S. Feingold, J. Jorgensen, G. S. Kobayashi, and S. Shadomy.** 1986. Results of a survey of antifungal susceptibility tests in the United States and interlaboratory comparison of broth dilution testing of flucytosine and amphotericin B. J. Clin. Microbiol. **23:**298–301.
5. **Casadevall, A., E. D. Spitzer, D. Webb, and M. G. Rinaldi.** 1993. Susceptibilities of serial *Cryptococcus neoformans* isolates from patients with recurrent cryptococcal meningitis to amphotericin B and fluconazole. Antimicrob. Agents Chemother. **37:**1383–1386.
6. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B **217**(Suppl.)**:**1–90.
7. **Espinel-Ingroff, A., C. W. Kish, Jr., 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.
8. **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.
9. **Galgiani, J. N.** 1993. Susceptibility testing of fungi: current status of the standardization process. Antimicrob. Agents Chemother. **37:**2517–2521.
10. **Galgiani, J. N., J. Reiser, C. Brass, A. Espinel-Ingroff, M. A. Gordon, and T. M. Kerkering.** 1987. Comparison of relative susceptibilities of *Candida* species to three antifungal agents as determined by unstandardized methods. Antimicrob. Agents Chemother. **31:**1343–1347.
11. **Ghannoum, M. A., A. S. Ibrahim, Y. Fu, M. C. Shafiq, J. E. Edwards, Jr., and R. S. Criddle.** 1992. Susceptibility testing of *Cryptococcus neoformans*: a microdilution technique. J. Clin. Microbiol. **30:**2881–2886.
12. **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.
13. **Pfaller, M. A., L. Burmeister, M. S. Bartlett, and M. G. Rinaldi.** 1988. Multicenter evaluation of four methods of yeast inoculum preparation. J. Clin. Microbiol. **26:**1437–1441.
14. **Pfaller, M. A., B. Dupont, G. S. Kobayashi, J. Müller, M. G. Rinaldi, A. Espinel-Ingroff, S. Shadomy, P. F. Troke, T. J. Walsh, and D. W. Warnock.** 1992. Standardized susceptibility testing of fluconazole: an international collaborative study. Antimicrob. Agents Chemother. **36:**1805–1809.
15. **Pfaller, M. A., M. G. Rinaldi, J. N. Galgiani, M. S. Bartlett, B. A. Body, A. Espinel-Ingroff, R. A. Fromtling, G. S. Hall, C. E. Hughes, F. C. Odds, and A. M. Sugar.** 1990. Collaborative investigation of variables in susceptibility testing of yeasts. Antimicrob. Agents Chemother. **34:**1648–1654.
16. **Price, M. F., M. T. LaRocco, and L. O. Gentry.** 1994. Fluconazole susceptibilities of *Candida* species and distribution of species recovered from blood cultures over a 5-year period. Antimicrob. Agents Chemother. **38:**1422–1424.
17. **Rex, J. H., C. R. Cooper, Jr., W. G. Merz, J. N. Galgiani, and E. J. Anaissie.** 1995. Detection of amphotericin B-resistant *Candida* isolates in a broth-based system. Antimicrob. Agents Chemother. **39:**906–909.
18. **Rex, J. H., M. A. Pfaller, M. G. Rinaldi, A. Polak, and J. N. Galgiani.** 1993. Antifungal susceptibility testing. J. Clin. Microbiol. Rev. **6:**367–381.
19. **Rodriguez-Tudela, J. L., and J. V. Martinez-Suarez.** 1994. Improved medium for fluconazole susceptibility testing of *Candida albicans*. Antimicrob. Agents Chemother. **38:**45–48.