JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1992, p. 1976–1981
0095-1137/92/081976-06$02.00/0

Vol. 30, No. 8

Collaborative Investigation of Broth Microdilution and Semisolid Agar Dilution for In Vitro Susceptibility Testing of *Candida albicans*

RIBHI SHAWAR,[1]† VICTOR PAETZNICK,[2] ZITA WITTE,[1] LISA GARNSEY ENSIGN,[3] ELI ANAISSIE,[2] AND MARK LAROCCO[1]*

Department of Pathology and Laboratory Medicine, University of Texas Medical School, Houston, Texas 77225,[1] and Department of Infectious Diseases[2] and Department of Biomathematics,[3] M. D. Anderson Cancer Center, Houston, Texas 77225

Received 10 February 1992/Accepted 4 May 1992

A study was performed in two laboratories to evaluate the effect of growth medium and test methodology on inter- and intralaboratory variations in the MICs of amphotericin B (AMB), flucytosine (5FC), fluconazole (FLU), itraconazole (ITRA), and the triazole Sch 39304 (SCH) against 14 isolates of *Candida albicans*. Testing was performed by broth microdilution and semisolid agar dilution with the following media, buffered to pH 7.0 with morpholinepropanesulfonic acid (MOPS): buffered yeast nitrogen base (BYNB), Eagle's minimal essential medium (EMEM), RPMI 1640 medium (RPMI), and synthetic amino acid medium for fungi (SAAMF). Inocula were standardized spectrophotometrically, and endpoints were defined by the complete absence of growth for AMB and by no more than 25% of the growth in the drug-free control for all other agents. Comparative analyses of median MICs, as determined by each test method, were made for all drug-medium combinations. Both methods yielded similar (±1 twofold dilution) median MICs for AMB in EMEM and RPMI, 5FC in all media, and FLU in EMEM, RPMI, and SAAMF. In contrast, substantial between-method variations in median MICs were seen for AMB in BYNB and SAAMF, FLU in BYNB, and ITRA and SCH in all media. Interlaboratory concordance of median MICs was good for AMB, 5FC, and FLU but poor for ITRA and SCH in all media. Endpoint determinations were analyzed by use of kappa statistical analyses for evaluating the strength of observer agreement. Moderate to almost perfect interlaboratory agreement occurred with AMB and 5FC in all media and with FLU in EMEM, RPMI, and SAAMF, irrespective of the test method. Slight to almost perfect interlaboratory agreement occurred with ITRA and SCH in EMEM, RPMI, and SAAMF when tested by semisolid agar dilution but not broth microdilution. Kappa values assessing intralaboratory agreement between methods were high for 5FC in all media, for AMB in BYNB, EMEM, and RPMI, and for FLU in EMEM, RPMI, and SAAMF. One laboratory, but not the other, reported substantial to almost perfect agreement between methods for ITRA and SCH in EMEM, RPMI, and SAAMF. Both laboratories reported poor agreement between methods for the azoles in BYNB. Discrepancies noted in azole-BYNB combinations were largely due to the greater inhibitory effect of these agents in BYNB than in other media. These results indicate that the semisolid agar dilution and broth microdilution methods with EMEM or RPMI yield equivalent and reproducible MICs for AMB, 5FC, and FLU but not ITRA and SCH.

The increased incidence of fungal infections and the development of newer antifungal agents, especially the synthetic azoles, has increased the demand for in vitro susceptibility testing with antifungal agents. Despite many efforts, such testing remains unstandardized. Several parameters known to influence test results and some inherent problems in the methodology make interpretation difficult. Results are unreliable because of great variability in the MICs obtained under different test conditions. Parameters such as the organism being tested, inoculum density, medium composition, pH, incubation time, and incubation temperature have all been shown to affect test results (3, 4, 7, 14, 16, 17, 19).

Several problems pertain to the method of testing and the antifungal agent being tested. In vitro tests with imidazoles and triazoles have shown considerable variations in MICs, depending on inoculum size, medium composition, and test format. Test results often correlated poorly with in vivo and/or clinical findings (20). Results from interlaboratory comparative studies revealed considerable lack of agreement. Of all the parameters investigated, it was shown that medium composition and inoculum size had marked effects on MIC determinations, particularly with the azoles (3, 13). Progress towards standardization was made when Pfaller et al. (17) reported that the adjustment of inoculum size by spectrophotometry resulted in the best correlation among laboratories. In another comparative study (18), these investigators found good interlaboratory agreement in the MICs obtained by broth macrodilution with RPMI 1640 (RPMI).

Another problem encountered is that of partial inhibition of growth with azoles. The lack of sharp endpoints (often referred to as "tailing" or "trailing" endpoints) makes interpretation difficult and is often seen when testing *Candida albicans* (15, 16). Recently, Gordon et al. (8) described a reproducible semisolid agar method for the determination of MICs of selected azole antifungal agents against yeasts and molds. Our preliminary evaluation of this method revealed that it compared favorably with broth microdilution susceptibility testing (12).

The present collaborative study was performed to evaluate the effect of test methodologies (broth microdilution

* Corresponding author.
† Present address: Department of Pathology, Baylor College of Medicine, Houston, TX 77030.

versus semisolid agar dilution) and medium composition on intra- and interlaboratory correlations of the MICs of five antifungal agents against 14 isolates of *C. albicans* tested in four different media. The results of these comparisons are the basis for this communication.

MATERIALS AND METHODS

Antifungal agents. Five antifungal agents were used in this study: amphotericin B (AMB; E. R. Squibb and Sons, Princeton, N.J.), flucytosine (5FC; Roche Laboratories, Nutley, N.J.), fluconazole (FLU; Pfizer, Groton, Conn.), itraconazole (ITRA; Janssen Pharmaceutica, Piscataway, N.J.), and the triazole Sch 39304 (SCH; Schering Plough Corp., Bloomfield, N.J.). Stock solutions of these antifungal agents were prepared from reagent-grade powders by use of deionized water as a solvent for 5FC and 100% dimethyl sulfoxide as a solvent for the other compounds. The initial stock concentrations were 1,600 mg/ml for 5FC and the azole compounds and 200 mg/ml for AMB. The maximum concentration of dimethyl sulfoxide in the first well of the dilution scheme was 4%.

Organisms. Fourteen isolates of *C. albicans* (seven from each of two laboratories) were used in this study. Six of the isolates were the same as those included in the recent study of Pfaller et al. (18): ATCC 64544, ATCC 64545, ATCC 64546, ATCC 64549, ATCC 64550, and ATCC 64553. The remaining isolates were recent clinical isolates. Each of these isolates was stored at −70°C in double-strength skim milk. Isolates were subcultured onto chocolate agar to determine purity and then subcultured onto Sabouraud dextrose agar prior to susceptibility testing. Each investigator used a subculture from the same original stock of organisms.

Susceptibility test procedures. (i) Media and buffers. Four different medium formulations were used in this study: buffered yeast nitrogen base (BYNB; Difco), Eagle's minimal essential medium (EMEM; Sigma), RPMI (Sigma), and synthetic amino acid medium for fungi (SAAMF; American Biorganics, Inc., North Tonawanda, N.Y.). Powders were dissolved in 0.085 M morpholinepropanesulfonic acid (MOPS) (pH 7.0). Media were then filter sterilized (cellulose nitrate membrane; pore size, 0.2 μm; Nalge Co., Rochester, N.Y.) and stored at 4°C.

(ii) Broth microdilution. Drugs were diluted in solutions of the various assay media. Broth microdilution susceptibility testing was performed as follows. Liquid medium containing the drug was mixed with drug-free medium and sterile deionized water at a ratio of 2:1:1 to provide a working solution for each drug (stock concentrations were 16 μg/ml for AMB and 64 μg/ml for all other agents). Ten twofold serial dilutions were made with the respective broths (concentration ranges were 8 to 0.008 μg/ml for AMB and 64 to 0.06 μg/ml for all other drugs). One hundred microliters of each drug dilution was dispensed into each of 8 wells representing a column in 96-well microtiter trays. Each of the wells in the last column was filled with 100 μl of drug-free medium to serve as a growth control. Microtiter trays were stored at −70°C, except for those containing AMB in BYNB, which were stored at 4°C.

(iii) Semisolid agar dilution. Semisolid agar dilution susceptibility testing was performed as described by Gordon et al. (8). In brief, a solution containing an appropriate dilution of drug was mixed with drug-free medium and sterile 2% molten agar (45°C) at a ratio of 2:1:1. One-milliliter samples of this semisolid agar preparation were dispensed into sterile 24-well polystyrene trays, which were stored at 4°C. Two wells per tray were filled with drug-free semisolid medium to serve as a growth control. Each tray contained two complete sets of drug-containing media, 11 dilutions per set.

(iv) Inoculum. Yeast inocula were prepared as described previously (17). In brief, isolates were subcultured to Sabouraud dextrose agar and incubated at 35°C for 48 h before being tested. Homogeneous suspensions were made in sterile 0.85% saline in round glass cuvettes (19 by 150 mm) and spectrophotometrically adjusted to a turbidity which would permit exactly 85% transmission of light at 530 nm. The suspensions were further diluted 1:20 in sterile saline to make working inocula. Standard colony count procedures were used to ensure colony counts in the range of 1×10^4 to 5×10^4 CFU/ml. Ten microliters of inoculum was added to each well of the semisolid agar plates and the broth microtiter trays, including the growth control wells.

(v) Inoculation and scoring. Inoculated plates and trays were sealed and incubated at 35°C for 24 h. To assess drug activity more precisely, we scored the growth in each well as recommended by the National Committee for Clinical Laboratory Standards Subcommittee on Antifungal Susceptibility Testing (18). Considering growth in the drug-free control well as 100%, we scored growth in the drug-containing wells as 4+, 3+, 2+, 1+, and 0, corresponding to >75 to 100%, >50 to 75%, >25 to 50%, >0 to 25% (slightly hazy turbidity for broth microdilution or a thin lawn of growth on agar for semisolid agar dilution), and 0% (optically clear) the growth in the control well. Consistent results were obtained when the endpoint for AMB was defined as the lowest concentration with which the growth score was 0, and when the endpoint for 5FC and the azoles was defined as the lowest concentration with which the growth score was no more than 1+. These criteria were used in defining the MICs.

Study design. Investigators from two different laboratories (referred to as laboratories M and H) participated in this study. Each investigator received numerically coded isolates. Written procedures for reagent preparation and semisolid agar dilution and broth microdilution susceptibility testing were the same for both laboratories. Each isolate was tested in duplicate. At the completion of the study, all data were collected for analysis of intralaboratory and interlaboratory agreement of endpoint interpretations as affected by growth medium or test method.

Statistical methods. The data were analyzed for strength of agreement among laboratories, test methods, and media by use of the kappa statistic for the measurement of observer agreement. The numerical values of this statistic range up to 1.0, with kappa = 1.0 indicating perfect agreement, kappa = 0 signifying no agreement, and negative values representing disagreement. Labels for strength of agreement assigned to corresponding ranges for kappa values were as follows (10, 21): <0.0, poor; 0.0 to 0.2, slight; 0.21 to 0.4, fair; 0.41 to 0.6, moderate; 0.61 to 0.8, substantial; and 0.81 to 1.0, almost perfect.

RESULTS

MIC_{50}s. Median MICs and ranges of MICs for 14 isolates of *C. albicans* are shown in Table 1. Median MICs obtained by both laboratories for 5FC were similar (equivalent or ±1 twofold dilution) with all test media, except BYNB, and by both test methods. Both laboratories also reported consistent results for AMB, 5FC, and FLU when tested in EMEM and RPMI by either method. However, MIC_{50}s (highest MIC for 50% of isolates) of AMB in SAAMF, as reported by both laboratories, were 4-fold higher with broth microdilution and

TABLE 1. Ranges of MICs and median MICs for 14 isolates of *C. albicans* in four media

Antifungal agent	Method	Laboratory	Range of MIC (median MIC), in μg/ml, in:			
			BYNB	EMEM	RPMI	SAAMF
AMB	Broth	H	0.25–0.5 (0.50)	0.5 (0.5)	0.5–1.0 (0.5)	2.0–8.0 (2.0)
		M	0.25–0.5 (0.5)	0.5 (0.5)	0.5 (0.5)	2.0–4.0 (2.0)
	Agar	H	0.5–1.0 (1.0)	0.25–0.5 (0.5)	0.25–0.5 (0.25)	8.0 (8.0)
		M	0.5–1.0 (1.0)	0.25–0.5 (0.5)	0.25–0.5 (0.25)	8.0 (8.0)
5FC	Broth	H	0.06–>64 (0.125)	0.125–>64 (0.125)	0.125–>64 (0.25)	0.125–>64 (0.25)
		M	0.06–>64 (0.125)	0.06–>64 (0.25)	0.125–>64 (0.25)	0.125–>64 (0.25)
	Agar	H	0.06–>64 (0.06)	0.06–>64 (0.125)	0.06–>64 (0.125)	0.125–>64 (0.25)
		M	0.06–>64 (0.5)	0.06–>64 (0.125)	0.06–>64 (0.125)	0.125–>64 (0.25)
FLU	Broth	H	1.0–>64 (1.0)	>64 (>64)	>64 (>64)	>64 (>64)
		M	2.0–>64 (4.0)	>64 (>64)	>64 (>64)	>64 (>64)
	Agar	H	1.0–>64 (8.0)	4.0–>64 (>64)	4.0–>64 (>64)	>64 (>64)
		M	64–>64 (>64)	>64 (>64)	>64 (>64)	>64 (>64)
ITRA	Broth	H	1.0–32 (2.0)	0.06–>64 (4.0)	1.0–>64 (4.0)	0.125–>64 (0.25)
		M	1.0–>64 (4.0)	>64 (>64)	>64 (>64)	0.5–>64 (>64)
	Agar	H	0.06–>64 (0.06)	0.06–>64 (0.125)	0.06–>64 (4.0)	1.0–>64 (>64)
		M	0.06–>64 (>64)	1.0–>64 (>64)	>64 (>64)	>64 (>64)
SCH	Broth	H	0.25–>64 (1.0)	0.25–(>64 (0.5)	0.06–>64 (1.0)	0.5–2.0 (0.5)
		M	32–>64 (>64)	64–>64 (>64)	64–>64 (>64)	16–>64 (>64)
	Agar	H	0.06–32 (2.0)	1.0–>64 (>64)	1.0–>64 (>64)	1.0–>64 (>64)
		M	2.0–>64 (>64)	>64 (>64)	>64 (>64)	1.0–>64 (>64)

8- to 32-fold higher with semisolid agar dilution, compared with results obtained in other media. In general, the azole drugs had higher MIC_{50}s in EMEM, RPMI, and SAAMF than in BYNB, regardless of the test method.

Interlaboratory agreement. Kappa values for the level of interlaboratory agreement of MIC results stratified by antifungal agent, test method, and medium are shown in Table 2 (strength-of-agreement labels are given above). With one exception, substantial to perfect agreement occurred with AMB and 5FC tested in all media by both methods. The exception was 5FC tested in BYNB by the semisolid agar method, for which only fair agreement (kappa = 0.53) occurred. Almost perfect agreement occurred with FLU tested in EMEM, RPMI, and SAAMF by both methods. However, FLU-BYNB combinations produced only fair (kappa = 0.34) and slight (kappa = 0.06) interlaboratory agreement when tested by broth microdilution and semisolid agar dilution, respectively. Although almost perfect agreement occurred with ITRA and SCH tested in SAAMF by the semisolid agar method (kappa = 0.91), levels of agreement were generally lower and more variable for these drugs with all other medium-test method combinations (kappa = −0.32 to 0.72). Overall, the highest level of interlaboratory agreement occurred with AMB and 5FC (average kappa = 0.96 and 0.92, respectively) and then with FLU (average kappa = 0.76). Low-level interlaboratory agreement occurred with ITRA and SCH (average kappa = 0.26 and 0.21, respectively). The average kappa value for all agents combined showed substantial interlaboratory agreement for RPMI with either method and for EMEM with the semisolid agar method, while agreement was almost perfect for SAAMF with the semisolid agar method.

Comparison between methods within each laboratory. Kappa values for the strength of agreement between the semisolid agar dilution and broth microdilution methods within each laboratory are shown in Table 3 (labels categorizing these values are given above). Both laboratories reported substantial to almost perfect agreement (kappa = 0.69 to 1) between methods for the following drug-medium combinations: AMB and 5FC in BYNB; AMB, 5FC, and FLU in EMEM and RPMI; and 5FC and FLU in SAAMF. Moreover, both laboratories reported fair or poor agreement (kappa = −0.14 to 0.28) between methods for FLU and ITRA in BYNB and AMB in SAAMF. In contrast, while laboratory M reported substantial to perfect agreement (kappa = 0.62 to 1) between methods for SCH in all media and for ITRA in all media except BYNB, laboratory H reported poor to slight agreement (kappa = − 0.32 to 0.06), with a tendency to record much lower broth microdilution MICs for these two drugs.

The highest level of agreement between the test methods with the media selected occurred with 5FC (average kappa = 0.95) and then with AMB, FLU, SCH, and ITRA, in that order (Table 3). For all agents tested, data from both laboratories combined showed substantial agreement between methods most often when EMEM and RPMI were used as growth media (average kappa = 0.73 and 0.79, respectively), despite a wide range of kappa values (−0.32 to

TABLE 2. Interlaboratory agreement of MIC results obtained by broth microdilution and semisolid agar dilution for 14 isolates of *C. albicans*

Antifungal agent (overall kappa value)	Method	Kappa value of observer agreement based on endpoint readings that were identical or varied by ±1 twofold dilution in[a]:				Avg kappa value
		BYNB	EMEM	RPMI	SAAMF	
AMB (0.96)	Broth	1.0	1.0	1.0	0.69	0.92
	Agar	1.0	1.0	1.0	1.0	1.0
	Avg	1.0	1.0	1.0	0.85	0.96
5FC (0.92)	Broth	0.91	0.91	1.0	1.0	0.95
	Agar	0.53	1.0	1.0	1.0	0.88
	Avg	0.72	0.95	1.0	1.0	0.92
FLU (0.76)	Broth	0.34	1.0	1.0	1.0	0.83
	Agar	0.06	0.81	0.91	1.0	0.69
	Avg	0.2	0.91	0.95	1.0	0.76
ITRA (0.26)	Broth	0.43	−0.04	0.15	0.06	0.15
	Agar	0.15	0.15	0.25	0.91	0.36
	Avg	0.29	0.06	0.2	0.48	0.26
SCH (0.21)	Broth	−0.13	−0.04	0.06	−0.32	−0.11
	Agar	−0.04	0.53	0.72	0.91	0.53
	Avg	−0.09	0.25	0.39	0.29	0.21
All agents (0.62)	Broth	0.56	0.56	0.64	0.48	0.55
	Agar	0.35	0.7	0.78	0.96	0.69
	Avg	0.42	0.63	0.71	0.72	0.62

[a] Based on the percentage of isolates for which similar MICs were obtained in both laboratories.

TABLE 3. Intralaboratory agreement of MIC results obtained by broth microdilution versus semisolid agar dilution for 14 isolates of *C. albicans*

Antifungal agent (avg kappa value)	Laboratory	Kappa value of observer agreement based on endpoint readings that were identical or varied by ±1 twofold dilution in[a]:				Avg kappa value
		BYNB	EMEM	RPMI	SAAMF	
AMB (0.7)	H	0.69	1.0	1.0	0.28	0.74
	M	0.79	1.0	1.0	−0.14	0.66
	Avg	0.74	1.0	1.0	0.07	0.7
SFC (0.95)	H	1.0	0.91	1.0	1.0	0.98
	M	0.72	1.0	1.0	1.0	0.93
	Avg	0.86	0.95	1.0	1.0	0.95
FLU (0.69)	H	−0.13	0.81	0.91	1.0	0.65
	M	−0.04	1.0	1.0	1.0	0.74
	Avg	−0.09	0.91	0.95	1.0	0.69
ITRA (0.26)	H	−0.23	0.32	−0.04	−0.04	−0.16
	M	−0.04	0.91	1.0	0.81	0.67
	Avg	−0.13	0.29	0.48	0.39	0.26
SCH (0.39)	H	−0.13	−0.04	0.06	−0.23	−0.09
	M	0.62	1.0	1.0	0.81	0.86
	Avg	0.25	0.48	0.53	0.29	0.39
All agents (0.60)	H	0.24	0.47	0.58	0.40	0.42
	M	0.41	0.98	1.0	0.70	0.77
	Avg	0.33	0.73	0.79	0.55	0.60

[a] Based on the percentage of isolates for which similar MICs were obtained by both test methods.

1). In contrast, average kappa values for SAAMF and BYNB reflected only moderate and fair agreement (average kappa = 0.55 and 0.33, respectively).

Effect of partial inhibition. All test wells exhibiting a growth score of 0, 1+, and 2+ were analyzed in an attempt to evaluate the tailing (or trailing) endpoints. For each isolate, the lowest concentration of a given drug at which a score of 0 could be detected was compared with the lowest concentration at which a score of 1+ could be detected. A difference of fourfold (or more) between the readings was considered significant.

No significant tailing was seen for AMB or 5FC in either laboratory, regardless of the medium, method, or endpoint used. Comparison between readings based on 0 and 1+ endpoints showed that tailing was not seen frequently for FLU with either method when EMEM, RPMI, or SAAMF was used, because growth in all wells, including that with the highest concentration of drug, appeared similar to that in the control well (i.e., most MICs were >64 μg/ml). A gradual reduction in growth was noted with increasing concentrations of FLU in BYNB for a large number of isolates in both laboratories (23 of 28 with broth microdilution and 9 of 28 with semisolid agar dilution). For both ITRA and SCH, the number of isolates for which tailing was observed was variable. There was no particular trend, except that the number of isolates exhibiting tailing was much lower in one laboratory with semisolid agar dilution for ITRA and SCH in SAAMF and with broth microdilution for ITRA in BYNB. Overall, isolates were noted to show tailing more often with broth microdilution than with semisolid agar dilution, regardless of the medium used.

In general, both laboratories agreed on the occurrence of tailing in a significant number of isolates for ITRA and SCH in all media, although the endpoints at which the tailing was noted were often variable between laboratories (e.g., 2 of 14 and 12 of 14 isolates exhibited tailing with 1+ endpoints in SAAMF with broth microdilution in laboratories M and H, respectively, while 11 of 14 and 4 of 14 isolates exhibited tailing with 2+ endpoints, respectively).

DISCUSSION

This study is the first interlaboratory comparison of broth microdilution and semisolid agar dilution with four media recently evaluated as candidates for antifungal susceptibility testing (18). To assess the correlation among laboratories, media, and methods, we analyzed ranges of MICs and median MICs of five antifungal agents against 14 isolates of *C. albicans*. In addition, kappa statistical analyses, designed to evaluate the strength of observer agreement, were used. This method is a chance-corrected measure of agreement that compares individual datum points and provides a statistic indicating the strength of the determined agreement. Hence, since kappa values compare the strength of the observed agreement with the strength of the agreement expected by chance alone, this measure of endpoint determination provides valuable information in addition to the ranges of MICs and median MICs.

With both methods and all media, only minor interlaboratory differences in the median MICs of AMB and 5FC occurred (Table 1). This result is also reflected by the observation of high kappa values for both of these antifungal agents (Table 2). However, this result is in sharp contrast to those of earlier studies of Calhoun et al. (4) and Galgiani et

al. (7), who reported up to several thousandfold differences in MICs (especially for 5FC) among laboratories. It is possible that the better correlation that we observed is due to a better standardization of the inoculum, well-defined interpretive guidelines, or simply the use of different test methods. The results of a recent collaborative study suggest that a better standardization of the inoculum and uniform performance of procedures can improve interlaboratory correlation (18). These authors reported that agreement among laboratories was significantly better for 5FC than for AMB, for which the results were more medium dependent; good interlaboratory agreement for AMB was observed with RPMI but not BYNB or SAAMF. In contrast, we observed good interlaboratory agreement for AMB with all media. This disparity may be attributed to differences in methodologies and/or the large number of participating laboratories in the aforementioned study, thus allowing an increased chance for discrepancies.

Uniform interlaboratory agreement could not be extended to the azoles. Results were dependent on the agent, medium, and method used. The lack of interlaboratory agreement between MICs was prominent with BYNB for all azoles, regardless of the test method (kappa = −0.13 to 0.43), and with SAAMF for ITRA and SCH with broth microdilution (kappa = 0.06 and −0.32, respectively). This lack of agreement was partly the result of tailing endpoints or partial inhibition, which was seen with BYNB over a wide range of drug concentrations. Agreement between azole MICs with other media was due to confluent growth in all wells, including those containing the highest drug concentration. Overall, the level of interlaboratory agreement, regardless of antifungal agent or test method, was substantial with EMEM (average kappa = 0.63), RPMI (average kappa = 0.71), and SAAMF (average kappa = 0.72) but moderate with BYNB (average kappa = 0.42).

Both test sites reported almost perfect intralaboratory correlation between broth microdilution and semisolid agar dilution for AMB, 5FC, and FLU tested in EMEM and RPMI (Table 3). The observation by laboratory M but not laboratory H of similar method-to-method correlations for ITRA and SCH in these media is most likely a reflection of differences in endpoint interpretation. This result illustrates the degree of subjectivity associated with this test parameter and the critical impact on test outcome.

Poor correlation between methods was observed by both laboratories for FLU and ITRA tested in BYBN and for AMB tested in SAAMF (Table 3). Furthermore, laboratory H reported low kappa values for ITRA and SCH tested in SAAMF. Method variability for FLU and SCH tested in SAAMF has been previously reported (13). These investigators found disagreement between broth microdilution and broth macrodilution when endpoints were determined visually. Disparity was greatly reduced, however, by agitation of microtiter trays followed by spectrophotometric determination of endpoints. Collaborators in this study (E.A. and V.P.) recently reported that agitation of microtiter trays resulted in clear-cut endpoints for FLU, as determined by visual inspection (1).

Recently, Espinel-Ingroff and coworkers (6) compared MICs obtained by microdilution versus macrodilution in RPMI. They found good correlation for 5FC and FLU but not AMB. AMB MICs were shown to be consistently higher in microdilution than in macrodilution. In contrast, we found no disparity in the results obtained by broth microdilution versus semisolid agar dilution for AMB in RPMI.

Overall, MIC_{50} data and kappa values indicate that the best agreement between methods occurred with EMEM and RPMI (average kappa = 0.73 and 0.79, respectively), regardless of the antifungal agent used or the laboratory in which the testing took place. Only fair to moderate agreement between methods was observed for MICs obtained in BYNB and SAAMF (average kappa = 0.33 and 0.55, respectively). Armelles (2) reported no significant difference in the MICs of ketoconazole, miconazole, and econazole determined in either BYNB or SAAMF by broth macrodilution. Some investigators previously showed that MICs were higher for AMB but lower for 5FC when determined in BYNB compared with other media (5). In contrast, using broth microdilution, we found the MIC_{50}s of AMB in BYNB to be two- to eightfold lower than those in other media, while the MIC_{50}s of 5FC were similar in all media. McIntyre and Galgiani (13) evaluated the effect of four media on broth macrodilution susceptibility for SCH and found variations in MICs ranging from 4- to 200-fold, with inconsistent differences in the MICs for six isolates of *C. albicans*. In the present study, the only significant variation in the MIC_{50}s of SCH (64- to 128-fold) occurred in EMEM with broth microdilution and in RPMI with semisolid agar dilution.

Partial inhibition of growth has been recognized as a major problem in the determination of endpoints when evaluating the in vitro activities of the azoles (9, 15, 16). Differences in the scoring of growth have resulted in variability of endpoint reporting and thus differences in MICs. In our evaluation, this phenomenon was noted by both laboratories, but the variations were contingent on the medium, antifungal agent, and/or method used. Tailing endpoints were most evident in BYNB over a wide range of concentrations and were expressed by a large number of isolates. In fact, the noticeable disagreement between results obtained in BYNB and those obtained in other media was largely due to the fact that confluent growth was noted at the highest drug concentration in the other media, resulting in uniform endpoints of >64 μg/ml. Therefore, it is difficult for us to determine which result is authentic, but this partial inhibition phenomenon needs to be addressed before standardized antifungal susceptibility testing can be realized.

The results of this preliminary investigation do not permit a clear recommendation of either broth microdilution or semisolid agar dilution as the method of choice for antifungal agent testing. Moreover, recent progress made by the National Committee for Clinical Laboratory Standards Subcommittee on Antifungal Susceptibility Testing suggests broth macrodilution to be the preferred method (4, 6, 14). There are, however, advantages to pursuing microtiter tray methods, as they are quite familiar to clinical microbiology laboratories, allow for large-scale production and storage, and lend themselves more easily to automation (11). The semisolid agar dilution format, although more difficult to prepare, is more suitable for susceptibility testing of molds (8).

We conclude that susceptibility testing of AMB, 5FC and, to a lesser extent, FLU by either broth microdilution or semisolid agar dilution can be standardized by selecting defined media and a standardized inoculum. Discrepancies with other azoles, however, remain a problem.

ACKNOWLEDGMENT

This work was supported in part by grant CA 16672 (Biostatistical Resource Group) from the National Institutes of Health.

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.
2. **Armelles, M.** 1989. Azole sensitivity in *Candida albicans*. FEMS Microbiol. Lett. **60:**153–158.
3. **Calhoun, D. L., and J. N. Galgiani.** 1984. Analysis of pH and buffer effects on flucytosine activity in broth dilution susceptibility testing of *Candida albicans* in two synthetic media. Antimicrob. Agents Chemother. **26:**364–367.
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. **Doern, G. V., T. A. Tubert, K. Chapin, and M. G. Rinaldi.** 1986. Effect of medium composition on results of macrobroth dilution antifungal susceptibility testing of yeasts. J. Clin. Microbiol. **24:**507–511.
6. **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.
7. **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.
8. **Gordon, M. A., E. W. Lapa, and P. G. Passero.** 1988. Improved method for azole antifungal susceptibility testing. J. Clin. Microbiol. **26:**1874–1877.
9. **Hughes, C. E., R. L. Bennett, and W. H. Beggs.** 1987. Broth dilution testing of *Candida albicans* susceptibility to ketoconazole. Antimicrob. Agents Chemother. **31:**643–646.
10. **Landis, J. R., and G. G. Koch.** 1977. The measurement of observer agreement for categorical data. Biometrics **33:**159–174.
11. **LaRocco, M.** 1991. Recent advances in antifungal susceptibility testing. Clin. Microbiol. Newsl. **13:**81–85.
12. **LaRocco, M. T., R. Shawar, and A. Wanger.** 1989. Comparison of semisolid agar dilution and broth microdilution for susceptibility testing of clinical yeast isolates against six antifungal agents, abstr. no. C-306, p. 444. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989.
13. **McIntyre, K. A., and J. N. Galgiani.** 1989. In vitro susceptibilities of yeasts to a new antifungal triazole, SCH39304: effects of test conditions and relation to in vivo efficacy. Antimicrob. Agents Chemother. **33:**1095–1100.
14. **National Committee for Clinical Laboratory Standards.** 1986. Antifungal susceptibility testing: committee report, vol. 5, no. 17. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Odds, F. C.** 1980. Laboratory evaluation of antifungal agents: a comparative study of five imidazole derivatives of clinical importance. J. Antimicrob. Chemother. **6:**749–761.
16. **Odds, F. C.** 1985. Laboratory tests for the activity of imidazole and triazole antifungal agents *in vitro*. Semin. Dermatol. **4:**260–270.
17. **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.
18. **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.
19. **Shadomy, S., and M. Pfaller.** 1991. Laboratory studies with antifungal agents: susceptibility tests and quantitation in body fluids, p. 1173–1183. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
20. **Warnock, D. A.** 1989. Antifungal drug susceptibility testing. Curr. Top. Med. Mycol. **3:**403–416.
21. **Woolson, R. F.** 1987. Statistical methods for the analysis of biomedical data, p. 252–260. John Wiley & Sons, Inc., New York.