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Influence of Inoculum Growth Phase on Microdilution Susceptibility Tests

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Two types of commercially available microdilution trays were inoculated with log-phase cultures, stationary-phase cultures, and direct suspensions of 18- to 24-h colonies, with essentially comparable results. Direct pick-up of colonies was also tested with two Prompt inoculation systems (3M Co.); they were found to be convenient and reliable methods for standardizing inocula without preincubation in broth and without turbidity adjustment.

Careful control of inoculum density is essential to obtain reasonable precision with any antimicrobial susceptibility test (3). Susceptibility tests are commonly performed with broth subcultures initiated with four or five colonies selected from primary agar plates. Standardization of the inoculum from microdilution tests is often performed by adapting procedures that were developed for standardizing the disk test, i.e., by adjusting a log-phase broth culture to match the turbidity of a McFarland 0.5 standard (7, 9, 11), or by appropriate dilution of a small volume (0.5 ml) broth culture that has been incubated for 4 to 6 h to reach the stationary phase of growth (2, 4, 9, 11). Both of the latter methods are commonly utilized for microdilution susceptibility testing. In the present report, we attempt to determine whether the physiological state of the cells in the initial inoculum actually influences the results of tests in two types of commercially available microdilution panels. In addition, we attempt to determine whether the incubation period can be dispensed with by simply preparing a saline suspension of colonies from an 18- to 24-h agar plate (stationary phase of growth) and then immediately standardizing by adjusting turbidity to match that of a McFarland 0.5 standard. The latter approach has proven to be satisfactory for performing disk diffusion susceptibility tests (6, 8).

The Prompt inoculation system (3M Co., St. Paul, Minn.) presents an alternative method for inoculum standardization without an incubation period and without visual turbidity adjustment (1, 13). The critical portion of this system is a polypropylene wand with cross-hatched grooves on one end. The grooves are designed to hold about 10^8 CFU when touched to five large (>1.0-mm) isolated colonies (10 colonies if they are 0.5 to 1.0 mm in diameter). Prompt no. 6306 contains 1.15 ml of saline (0.85% NaCl) and is designed to provide an inoculum density equal to that obtained with a McFarland 0.5 turbidity standard. Prompt no. 6306 was developed for disk diffusion tests, but would also be appropriate for preparing the rehydrating inoculum utilized for the Sceptor dried microdilution panels (BBL Microbiology Systems, Cockeysville, Md.) (10). Another Prompt system (no. 6309) contains 30 ml of saline (0.85% NaCl). This product is designed to provide an inoculum density suitable for inoculating frozen microdilution panels, such as those provided by Micro-Media Systems (MMS; San Jose, Calif.) (5). Both of the above Prompt systems were evaluated in the current study. Thirty-milliliter Prompt units with water containing Tween 80 or with cation supplements were not studied.

The primary purpose of this study was to determine whether minimal inhibitory concentrations (MICs) would be influenced by the physiological state of the bacteria cells in five different types of inocula. To provide protocol control, random viable cell counts were also performed from the inoculated growth control wells and from the standardized cell suspensions.

MATERIALS AND METHODS

Bacterial strains. Tests were performed with 20 selected isolates, 17 of which produced β-lactamase as evidenced by nitrocefin hydrolysis with or without prior induction. Three standard control strains (11, 12) were included, i.e., *Staphylococcus aureus* (ATCC 29213), *Streptococcus faecalis* (ATCC 29212), and *Pseudomonas aeruginosa* (ATCC 27853). Also tested were four additional *S. aureus*, three *Klebsiella pneumoniae*, two *Escherichia coli*, two *Enterobacter agglomerans*, one *Enterobacter cloacae*, one *Citrobacter diversus*, one *Providencia stuartii*, one *Providencia rettgeri*, one *Morganella morganii*, and one *Acineto-*

bacter calcoaceticus var. *anitratus*. Each strain was transferred on blood agar for 2 consecutive days before testing and was subsequently tested on 6 separate days. Each day, four MMS frozen panels and four Sceptor dried panels were inoculated, each with a different type of inoculum.

Inoculum standardization procedures. Five different inoculum standardization procedures were evaluated: the first three were used to prepare inocula for both types of panels.

(i) Log phase. Actively growing (2- to 4-h) trypticase soy broth (BBL) cultures were adjusted by adding saline until the turbidity matched that of a McFarland 0.5 standard, as judged by viewing the cell suspension and turbidity standard against a white background with black lines and a standard light source (7, 11, 12).

(ii) Stationary phase. Four to five colonies were transferred to 0.5 ml of brain heart infusion broth; after 5 to 6 h at 35°C, the culture was assumed to be in the stationary phase of growth. Such cultures have been found to contain ca. 10^9 CFU/ml (2, 4). Consequently, a simple 1:10 dilution should yield an inoculum comparable to that achieved with the first method.

(iii) Direct suspension. Colonies were selected from 18- to 24-h blood agar plates and suspended in 1.0 ml of saline. After mixing with a Vortex mixer, the suspension was adjusted to match the turbidity of a McFarland 0.5 standard, as described above for the log phase.

(iv) Prompt no. 6306 (1.15 ml). Five isolated colonies ≥1 mm in diameter were touched with the inoculation wand, suspended in the unit's 0.85% NaCl, and then mixed in a Vortex mixer. This system was used only for inoculating Sceptor panels, which require 10 μl of a suspension containing ca. 10^8 CFU/ml.

(v) Prompt no. 6309 (30 ml). With the same direct pickup described above, the charged wand was immersed into the unit's 30 ml of NaCl solution and shaken. This cell suspension was then poured into an MMS inoculum tray, and an MMS test panel was inoculated by using a disposable inoculum transfer unit that accompanies the system (6).

Microdilution test panels. Frozen panels from MMS were held at −20°C or lower and were allowed to thaw at room temperature just before use. Thirty milliliters of sterile water was inoculated with 0.3 ml of a suspension that should contain 10^8 CFU/ml, i.e., procedures i and iii. With method ii, 0.03 ml was transferred to 30 ml of sterile water. The method v suspension was used without further dilution. This inoculum was then poured into a seed tray, and a disposable inoculum transfer device was used to inoculate the wells in the thawed test panel. This device should deliver approximately 5 μl of inoculum to each well containing approximately 0.1 ml of broth.

Dried Sceptor panels were inoculated and rehydrated by delivering approximately 0.1 ml of seeded broth to each well by using a semiautomated inoculator. This device uses 10 ml of a modified Mueller-Hinton broth seeded with a 10-μl disposable calibrated loopful of a suspension containing ca. 10^8 CFU/ml. In sampling the standardized cell suspensions, care was taken to be certain that the disposable calibrated loop actually transferred a loopful of liquid.

Once inoculated, both types of test panels were incubated for 16 to 18 h at 35°C. At that time MICs were recorded as the lowest concentration that completely inhibited visible growth of the microorganisms. With MMS panels, 13 MICs were provided for gram-negative bacilli, and 11 MICs were available for gram-positive cocci. With Sceptor panels, 12 MICs were provided for both types of microorganisms (different drugs).

Viable cell counts. The number of viable cells actually delivered to the microdilution trays was determined by removing 25 μl from the growth control well immediately after inoculation. This sample was transferred to 2.5 ml of sterile saline, and 0.1 ml of that dilution was plated onto a Trypticase soy agar plate in a thin agar overlay (2, 4). Colonies were counted after 24 h at 35°C and periodically recounted after 48 h. For each strain eight microdilution panels were inoculated on 1 day, and the inocula delivered to all eight panels were estimated. The inocula delivered to the eight different panels were determined on 6 separate days when the three control strains were being tested, but on only 1 of the 6 days with the other strains. On one occasion viable cell counts were also performed with the five different types of standardized cell suspensions before the panels were inoculated with the three control strains.

RESULTS

Inoculum standardization. Viable cell counts were obtained from the five different types of standardized cell suspensions (Table 1). The three control strains provided inocula ranging from 3×10^7 to 3×10^8 CFU/ml (1×10^6 to 5×10^6 CFU/ml with 30-ml Prompt units). With the gram-positive cocci, the first four methods provided essentially identical inocula. However, with *P. aeruginosa*, stationary-phase cultures and direct colony suspensions yielded colony counts lower than those obtained with log-phase cultures. Presumably, either the older cultures contain a significant number of nonviable cells that contribute turbidity to a cell suspension or clumping of the older cells tended to provide erroneously low colony counts.

Inoculum density. The actual number of viable cells delivered to wells in the microdilution panels is summarized in Table 2. These values are dependent upon the number of viable cells in the standardized cell suspensions and on the actual volume of inoculum delivered to the growth control well of each test panel. The disposable loops provided with the Sceptor system were monitored carefully to be certain that the loops were fully charged before seeding the broth medium. A significant proportion (ca. 10%) of the loops were discarded because they failed to perform satisfactorily.

Exceptionally low colony counts were obtained with the control strain of *P. aeruginosa*. However, MIC values were all within the expected range (12) for that strain. Repeated colony counts with surface streaking and prolonged incubation failed to yield higher cell counts. We concluded that some unexplained factors were

TABLE 1. Viable cell counts achieved with five different methods of inoculum standardization, before inoculation of microdilution susceptibility test trays

Standardization procedure	Mean of duplicate counts (CFU/ml)		
	P. aeruginosa (ATCC 27853)	*S. faecalis* (ATCC 29212)	*S. aureus* (ATCC 29213)
Log-phase broth culture adjusted to McFarland 0.5[a]	2.4×10^8	7.9×10^7	6.5×10^7
Stationary-phase broth culture diluted 1:10[a]	4.1×10^7	1.3×10^8	1.3×10^8
Direct suspension of colonies adjusted to McFarland 0.5[a]	3.0×10^7	2.6×10^8	9.2×10^7
Direct pickup of colonies in 1.0-ml Prompt[a]	4.5×10^7	6.0×10^7	1.8×10^8
Direct pickup of colonies in 30-ml Prompt[b]	2.2×10^6	1.1×10^6	5.3×10^6

[a] Expected cell density, 1×10^8 (2×10^7 to 2×10^8) CFU/ml.
[b] Expected cell density, 3×10^6 (7×10^5 to 7×10^6) CFU/ml.

affecting our cell counts with this strain, but not with other species. The tendency for *P. aeruginosa* to clump is the most likely explanation, especially when the cells are in the stationary phase of growth. The data in Table 2 exclude tests with the *P. aeruginosa*. Mean viable cell counts with MMS trays approached the expected level of 10^5 CFU/ml. Direct colony suspensions for MMS panels tended to yield significantly ($P \leq 0.05$; rank sum test) lower inocula with gram-negative bacilli, but not with gram-positive cocci. With all four systems, 95% confidence limits for tests with gram-negative bacilli varied from 10^4 to 10^6 CFU/ml. With the Sceptor system, inocula tended to be lower than those calculated for MMS panels (1×10^3 to 5×10^5 CFU/ml). Presumably, the disposable calibrated loop used to seed the Sceptor broth medium was not delivering 10 μl of the standardized cell suspension. With all four procedures, some exceptionally low inocula ($<5 \times 10^4$ CFU/ml) were observed.

Reproducibility of MICs. Intrasystem reproducibility of MICs was first examined by calculating the ranges of MIC values recorded when six separate tests were performed (Table 3). Reproducibility of the four inoculum standardization procedures was quite comparable. A range of only one dilution step was seen with 90 to 98% of all the different sets of six MICs. MICs varied ± 1 $\log_2$ dilution interval (range of two dilution steps) with 97 to 100% of the data sets.

Direct comparison of paired MICs. Table 4 shows a direct comparison of the different methods of inoculum standardization in terms of comparability of MIC values obtained in parallel

TABLE 2. Ranges of viable cell counts from samples removed from growth control wells immediately after inoculation of frozen or dried microdilution panels, each with four different types of standardized inocula

Inoculum standardization method	CFU/ml of culture			
	Gram-negative bacilli[a]		Gram-positive cocci	
	Mean	95% Confidence limits	Mean	95% Confidence limits
MMS frozen panels				
Log phase	1.1×10^5	2.2×10^4 to 5.7×10^5	7.4×10^4	3.1×10^4 to 1.7×10^5
Stationary phase	1.9×10^5	5.2×10^4 to 7.2×10^5	1.5×10^5	9.5×10^4 to 2.5×10^5
Direct suspension	7.6×10^4	1.1×10^4 to 5.1×10^5	1.1×10^5	4.2×10^4 to 2.9×10^5
Prompt (30 ml)	2.5×10^5	5.9×10^4 to 1.0×10^6	2.0×10^5	7.8×10^4 to 5.2×10^5
Sceptor dried panels				
Log phase	5.4×10^4	1.4×10^4 to 2.1×10^5	2.7×10^4	1.1×10^4 to 6.6×10^4
Stationary phase	6.3×10^4	1.7×10^4 to 2.4×10^5	4.8×10^4	2.5×10^4 to 9.1×10^4
Direct suspension	2.1×10^4	1.5×10^3 to 2.9×10^5	1.0×10^5	2.0×10^4 to 5.1×10^5
Prompt (1.0 ml)	3.0×10^4	5.0×10^3 to 1.8×10^5	5.7×10^4	1.3×10^4 to 2.5×10^5

[a] Excludes replicate tests with the *P. aeruginosa* since it yielded unusually low colony counts, possibly due to difficulties in the counting technique. The means of six colony counts with the *P. aeruginosa* ranged from 4.1×10^3 (direct) to 5.6×10^4 (log phase) in MMS panels and from 6.3×10^3 (direct) to 5.9×10^4 (log phase) in Sceptor panels. The two types of Prompt systems yielded mean counts of 3.5×10^4 (MMS panels) and 8.7×10^3 (Sceptor panels).

TABLE 3. Intrasystem reproducibility of MICs with different inoculum standardization methods

Inoculum standardization method	Range of 6 MICs in $\log_2$ dilution steps[a]							
	Gram-negative bacilli				Gram-positive cocci			
	0	≤1	≤2	≤3	0	≤1	≤2	≤3
MMS frozen panels								
Log phase	53.3	90.1	97.8	99.5	66.7	96.9	98.5	98.5
Stationary phase	62.6	90.7	98.3	99.5	78.8	97.0	100.0	
Direct suspension	67.6	96.1	100.0		77.3	93.9	97.0	100.0
Prompt (30 ml)	60.4	91.2	100.0		77.3	98.5	100.0	
Sceptor dried panels								
Log phase	55.9	94.1	98.8	99.4	62.5	94.4	100.0	
Stationary phase	49.4	92.3	99.4	100.0	54.2	95.8	100.0	
Direct suspension	61.3	90.5	98.8	100.0	51.4	94.4	100.0	
Prompt (1.0 ml)	55.4	91.7	98.2	99.4	58.3	93.1	100.0	

[a] Presented as cummulative percentages of strains with six separate MICs ranging 0, 1, 2, or 3 dilution intervals; no set of MICs varied more than 4 $\log_2$ dilution intervals.

tests. Identical MICs were observed with 73 to 87% of the paired MICs, and 97 to 99% of the paired MICs varied no more than one $\log_2$ dilution interval. Although the MICs were essentially comparable (within the limits of reproducibility of the tests), there was a general trend for some systems to give higher MICs (Table 4). Both of the above analyses include all MICs, even if they were off scale (less than or equal to the lowest concentration tested or greater than the highest concentration tested).

Comparison of on-scale MICs. Further analysis of these data was performed with those microorganism-drug combinations that produced on-scale endpoints with at least five of the six replicate tests. The nonparametric Friedman rank sum test was used to determine significance of differences between geometric means. With MMS panels, 52 microorganism-drug combinations were available for comparison; six displayed some significant differences ($P \leq 0.10$). With the Sceptor system, 66 combinations could be compared; five gave some significant ($P \leq 0.10$) differences. The 11 combinations with significant differences are described in Table 5. Only 5 of the 11 combinations had differences with $P \leq 0.05$. Highly significant ($P \leq 0.001$) differences were seen when *S. aureus* was tested against penicillin or ampicillin in MMS trays. In that situation, Prompt produced significantly higher MICs. The same level of significance was not observed when *S. aureus* was tested against the penicillins in Sceptor panels.

Table 6 shows a summary of data accumulated with the weak β-lactamase-producing control strain of *S. aureus* (ATCC 29213). With this

TABLE 4. Direct comparison of all paired MIC values obtained with different methods of inoculum standardization; includes MIC values that are off scale

Methods compared[a]	% of paired MIC values with 0 or ≤1 doubling dilution differences							
	Gram-negative bacilli				Gram-positive cocci			
	MMS panels (n = 1,089)		Sceptor panels (n = 1,008)		MMS panels (n = 396)		Sceptor panels (n = 432)	
	0	≤1	0	≤1	0	≤1	0	≤1
Log phase versus stationary phase	79.7	98.0	76.0	98.6[b]	85.9	98.7[c]	79.9	97.7
Stationary phase versus direct suspension	77.7	98.0[b]	73.5	97.8[b]	86.6	99.2[b]	76.6	97.7[c]
Log phase versus direct suspension	76.6	97.8[b]	73.2	97.6[b]	87.1	98.2[c]	78.9	99.3[c]
Prompt versus direct suspension	78.9	98.2[b]	79.8	98.0[b]	85.1	99.2[b]	81.5	99.3[c]
Prompt versus stationary phase	78.6	98.3	77.1	98.0[c]	85.4	99.7[b]	80.3	97.0
Prompt versus log phase	75.9	97.5	75.8	97.8[c]	79.8	98.7[b]	81.0	98.4

[a] See the text for detailed description of the methods being compared.
[b] Significant trend for higher MICs with the first method listed.
[c] Significant trend for lower MICs with the first method listed.

TABLE 5. Geometric mean MICs obtained with four methods of inoculum standardization; microorganism-drug combinations with any significant ($P \leq 0.10$) differences between methods

Microorganism	Drug	n[a]	Test panel	Geometric mean MIC (μg/ml)				P[b] (Friedman rank sum)
				Log phase	Stationary phase	Direct suspension	Prompt	
P. aeruginosa	Tetracycline	6	MMS	4.00	4.50	1.78	4.00	≤0.05
K. pneumoniae	Kanamycin	18	Sceptor	1.65	1.59	1.08	1.21	≤0.05
K. pneumoniae	Cefamandole	18	MMS	4.86	3.84	2.95	3.84	≤0.10
M. morganii	Cefamandole	6	Sceptor	20.11	18.00	10.06	10.06	≤0.10
Providencia sp.	Tobramycin	12	MMS	3.53	3.12	2.00	2.39	≤0.10
E. coli	Kanamycin	12	Sceptor	4.23	3.78	2.51	3.78	≤0.10
E. coli	Amikacin	12	MMS	4.00	3.18	2.68	4.50	≤0.10
S. aureus	Chloramphenicol	30	Sceptor	5.03	4.59	6.06	4.69	≤0.10
S. aureus	Penicillin	30	Sceptor	0.59	0.83	0.71	0.66	≤0.05
S. aureus	Penicillin	29	MMS	0.55	0.81	0.65	1.00	≤0.001
S. aureus	Ampicillin	30	MMS	0.97	1.32	1.07	1.52	≤0.001

[a] Number of MIC values for each method, six tests for each strain (one to five strains tested).

[b] Probability that all four methods yield equal MIC values. This table lists only microorganism-drug combinations with any significant differences. Tests with 46 combinations demonstrated no significant differences with MMS frozen panels, and 61 combinations demonstrated no significant differences with dried Sceptor panels. The other combinations were excluded because off-scale endpoints did not permit calculation of geometric means.

strain, Prompt generally tended to produce the largest inoculum, and log-phase cultures provided the smallest inoculum. Consequently, penicillin and ampicillin MICs tended to be higher when MMS panels were inoculated with the Prompt units. With the Sceptor panels, the inocula were somewhat lower, and all four systems yielded essentially comparable results. The differences between Sceptor and MMS MICs may simply reflect differences between the broth media used in these two systems. It would appear that the *S. aureus* strains tend to grow somewhat better in the Sceptor broth, even with a significantly lighter inoculum. MICs that could be misinterpreted as indicating susceptibility (MIC, ≤0.12 μg/ml were noted only with log-phase broth cultures (11, 12). The Prompt systems were least likely to lead to such misinterpretation with *S. aureus*. Tests with four other β-lactamase-producing strains of *S. aureus* provided similar data, i.e., lowest MICs with log-phase inocula and comparable results with MMS versus Sceptor panels.

DISCUSSION

The data presented in this report lead us to conclude that the physiological state of the cells (log phase versus stationary phase) does not significantly influence the results of broth dilution susceptibility tests. Although there was general agreement between MICs obtained with

TABLE 6. Penicillin and ampicillin MICs with a weak β-lactamase-producing control strain of *S. aureus* (ATCC 29213)

Inoculum standardization method	Inoculum (CFU/ml) in control wells[a]		MIC (μg/ml)			
	Mean	Range	Penicillin		Ampicillin	
			Mode	Range	Mode	Range
MMS frozen panels						
Log phase	7.1×10^4	4.8×10^4–9.3×10^4	0.25	0.12–0.25	0.5	0.5–0.5
Stationary phase	1.6×10^5	1.1×10^5–2.0×10^5	0.25	0.25–0.25	0.5	0.5–1.0
Direct suspension	1.2×10^5	8.3×10^4–1.6×10^5	0.25	0.25–0.25	0.5	0.5–1.0
Prompt (30 ml)	2.5×10^5	1.7×10^5–3.5×10^5	0.5	0.5–0.5	1.0	1.0–1.0
Sceptor dried panels						
Log phase	3.3×10^4	1.7×10^4–4.4×10^4	0.5	0.25–0.5	0.5	≤0.25–0.5
Stationary phase	5.4×10^4	3.0×10^4–6.9×10^4	0.5	0.25–0.5	0.5	0.5–0.5
Direct suspension	2.7×10^4	2.4×10^4–1.9×10^5	0.5	0.25–0.5	0.5	0.5–0.5
Prompt (1.0 ml)	8.6×10^4	5.3×10^4–1.2×10^5	0.5	0.5–0.5	0.5	0.5–1.0

[a] Each system was tested on six separate occasions, and viable cell counts were performed on samples from the growth control wells immediately after inoculation. All inocula were adjusted to yield a theoretical inoculum of 10^5 CFU/ml.

different types of inocula, there were significant trends for slightly higher or lower MICs when different methods were compared directly. Such minor discrepancies can be attributed to differences in the inoculum density rather than differences in the physiological state of the cells in the inoculum. Inocula obtained from colonies older than 24 h or from overnight broth cultures were not evaluated in this study.

Reproducibility of MICs was remarkable, considering the marked variability in the inoculum density that was estimated from colony counts. It is not possible to determine whether our widely varied inoculum estimates actually represent inprecision in the counting procedure or inprecision in the methods of inoculating the trays or inprecision in the methods for standardizing the cell suspension. The limited data presented in Table 1 would seem to eliminate the latter source of variability. Our apparent difficulties in obtaining useful inoculum estimates with the *P. aeruginosa* suggest that our colony counts might be a little on the low side, at least with some strains. We assume that the low counts with *P. aeruginosa* might be attributed to the organism's tendency to form clumps of cells. That problem was not investigated; our primary goal was to compare MIC values and not colony counts. MICs with *P. aeruginosa* all fell within the expected range (12), except when aminoglycosides were tested in MMS trays without cation supplements.

Variability in the amount of inoculum transferred to the microdilution trays could be attributed to the inoculum transfer device used for the MMS trays or the disposable calibrated loop used for the Sceptor system. The data in Tables 1 and 2 suggest that the calibrated loop tends to deliver significantly less than 0.01 ml to the broth medium. The lot of loops that was used in this study frequently failed to fill completely; thus, it is entirely possible that these loops were a source of significant variability. That might explain why Sceptor panels tended to produce significantly lower colony counts, compared with MMS trays. It is also highly unlikely that the MMS inoculum transfer device actually delivers exactly 5 μl to every well.

It is often assumed that the inoculum can vary over one $\log_{10}$ range without greatly influencing most MICs. Although we attempted to maintain inocula between 2×10^4 and 2×10^5, some counts were outside of the range. It might be more appropriate to standardize the inoculum at a median of 5×10^5 (1×10^5 to 1×10^6) CFU/ml (12). With the current state of the art, an inoculum of 2×10^4 to 1×10^6 CFU/ml, i.e., the range achieved in this study, may be a more realistic goal.

Microdilution trays may be inoculated by adjusting turbidity of a log-phase broth culture or of a suspension of colonies selected from an overnight agar plate. The need for visual adjustment of turbidity may be avoided by using small-volume (0.5-ml) BHI broth cultures that have been incubated for at least 5 h. In the clinical laboratory, the latter technique is practical only for those cultures that can be initiated early in the morning. For those tests that need to be initiated later in the afternoon, the Prompt system provides an acceptable alternative that requires no incubation time or visual adjustment of turbidity. In practice, the latter manipulation is rarely performed as carefully as it should be. MIC values obtained with the Prompt systems were essentially comparable to those obtained with the other procedures. When *S. aureus* was tested against the penicillins, MICs tended to be a little higher, and inocula were somewhat greater. Difficulties can be encountered in separating penicillin-resistant and -susceptible staphylococci when a light inoculum is utilized. Tests with a larger series of *S. aureus* isolates will be needed to determine whether the Prompt system differs from alternative methods in this respect.

We concluded that the direct pickup approach provided by the Prompt system is a perfectly acceptable alternative method for standardization of inoculum for microdilution susceptibility tests. Because it requires no incubation period and no adjustment of turbidity, it represents a system which is more practical than the alternative standard methods described in this report.

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