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Reader Error in Determining Minimal Inhibitory Concentrations with Microdilution Susceptibility Test Panels

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Microdilution susceptibility test panels were independently read by two different technologists. One or more discrepancies between readers occurred with 5% of the 25,022 recorded minimal inhibitory concentrations, but only 0.4% of the tests varied by ≥2 dilution intervals.

Microdilution technology has made it possible for clinical laboratories routinely to perform quantitative antimicrobial susceptibility tests. For each antimicrobial agent, different concentrations (usually twofold dilutions) are prepared and dispensed into different wells of a microdilution tray. The trays may be frozen until needed and then allowed to thaw just before inoculation with a standardized suspension of the test strain. After 18 to 24 h at 35°C, the trays are examined, and for each drug, a minimal inhibitory concentration (MIC) is defined as the lowest concentration which completely inhibits microbial growth, overlooking a very faint haze or tiny button that can be observed only with difficulty. With some antimicrobial agent-microorganism combinations, subinhibitory concentrations of drug may markedly reduce but not completely inhibit microbial growth. Consequently, it is occasionally difficult to determine whether or not the microorganism is completely inhibited by concentrations near the MIC. The actual MICs are affected by the optical arrangement used to observe the test panels and by the visual acuity of the individual reading the tests. When the endpoints are not well defined, different individuals observing the same test panel might record different MICs, usually varying by one dilution interval, occasionally differing by two or more dilution intervals.

In our clinical laboratory, microdilution susceptibility tests are performed routinely (1). Our MT (ASCP)-registered medical technologists rotate responsibility for reading the test results. When the test system was first initiated, all trays were read by two different technologists. Each morning, one technologist observed the test panels on a standard viewer (Dynatech Laboratories, Alexandria, Va.). The endpoints were selected and read to a recorder, who noted the results on a work card. The trays were then immediately examined by a second technologist, and the second reading was recorded on the same work card. When the two readings disagreed, arbitration was required to select the endpoint that was to be reported. This system of double reading minimized clerical error inherent in all manual recording systems and reduced the variability in defining certain "trailing" endpoints. It also served as an excellent training program to standardize the reading process among our technologists.

Because the process of double reading involves an additional expenditure of personnel time, we reviewed our laboratory records to determine whether a significant number of errors were being detected. All tests performed in a 2-month period were reviewed, excluding those that failed to grow in the growth control well or those that appeared to contain more than one microorganism (purity control).

A total of 2,426 test panels were examined by two observers (not always the same technologists). The two readers disagreed on the interpretation of one or more MICs with 31% of these test trays (Table 1). Discrepancies between readers were observed with all groups of microorganisms. Each microdilution tray contained either 9 drugs (gram positive) or 11 drugs (gram negative). Consequently, tests with the 2,426 strains generated 25,022 MICs. Arbitration of 5.2% of the MICs was necessary because the two readers disagreed. Almost all of the discrepancies involved differences of only one dilution interval, and only 0.4% of the tests involved differences of two or more dilution steps. This approaches a level of reproducibility that is generally considered satisfactory for tests involving serial twofold dilutions. Most discrepancies of ≥2 dilution steps represented differences in the interpretation of trailing endpoints. Occasionally such discrepancies represented clerical errors in recording the initial results, but that was relatively uncommon.

The different antimicrobial agents that were tested are listed in Table 2. The gram-positive

TABLE 1. *Discrepancies between two observers examining the same microdilution susceptibility test panels*

Microorganism	No. of tests examined		No. of MICs reported		
	Total no. of tests	No. (%) with ≥1 discrepant MICs	Total no. of MICs	No. (%) discrepant	
				1 dilution	≥2 dilutions
Staphylococcus	625	161 (26)	5,625	191 (3)	26 (0.5)
Enterococcus	207	71 (34)	1,863	94 (5)	0
Escherichia	545	240 (44)	5,995	303 (5)	38 (0.6)
Klebsiella	240	104 (43)	2,640	144 (5)	6 (0.2)
Enterobacter	101	65 (64)	1,111	84 (8)	1 (0.1)
Serratia	96	28 (29)	1,056	25 (2)	4 (0.4)
Citrobacter	30	5 (17)	330	8 (2)	0
Proteus-Providencia	146	53 (36)	1,606	66 (4)	5 (0.3)
Acinetobacter	66	23 (35)	726	30 (4)	1 (0.1)
Pseudomonas					
P. aeruginosa	265	157 (59)	2,915	192 (7)	26 (0.9)
Other spp.[a]	74	50 (68)	814	55 (7)	6 (0.7)
Miscellaneous[b]	31	8 (26)	344	10 (3)	0

[a] Includes 26 *P. maltophilia*, 2 *P. cepacia*, and 46 unidentified species.

[b] Includes 11 *Moraxella* spp., 7 *Aeromonas hydrophila*, 3 *Achromobacter xylosoxidans*, 2 *Alkaligenes* spp., 4 *Flavobacterium* spp., 3 *Pasteurella multocida*, and 1 *Bacillus* sp.

TABLE 2. *Antimicrobial agents with discrepant MICs recorded by two independent observers*

Antimicrobial agent	% Discrepant MICs	
	Gram-negative panels (n = 1,594)	Gram-positive panels (n = 832)
Erythromycin	NT[a]	2.0
Clindamycin	NT	4.6
Penicillin	NT	3.2
Ampicillin	3.8	3.5
Cephalothin	4.3	1.9
Tetracycline	9.8	7.2
Chloramphenicol	6.8	9.7
Gentamicin	7.3	2.8
Tobramycin	6.9	3.6
Amikacin	6.3	NT
Carbenicillin	3.8	NT
Nitrofurantoin	4.0	NT
Trimeth/Sulfa	3.8	NT
Sulfisoxazole	6.1	NT

[a] NT, Not tested.

panels contained two aminoglycosides because, at that time, we were interested in determining the incidence of gentamicin-resistant staphylococci; such results were recorded but not reported. Sulfisoxazole was included in the gram-negative panels; dilutions were prepared in the synthetic amino acid broth medium of Hoeprich et al. (2) (GIBCO Laboratories, Grand Island, N.Y.) to reduce the antagonists present in other media. With the sulfonamides and trimethoprim-sulfamethoxazole, the endpoint was defined as an 80% inhibition of growth compared to the growth control wells, i.e., a faint haze or small button of growth was considered negative (4). Discrepancies between readers were not unusually frequent with these antimicrobial agents; reader error was somewhat more common with tetracycline, chloramphenicol, and the aminoglycosides.

Difficulties in defining certain endpoints can definitely affect the precision of microdilution susceptibility tests. Clerical errors, which occur with any system involving manual transcription of laboratory data, may also affect precision (3). Automation and mechanization of the reading process should help to control such sources of variability. Automated systems are currently being developed but, at this time, most laboratories utilize manual systems for reading MICs and, consequently, some degree of variability must be accepted. The subjective error inherent in the current manual process should be appreciated, as should the potential for error in transcribing test results. In our laboratory, experienced, trained technologists were capable of demonstrating a satisfactory degree of precision in reading the test results after an initial training period of double reading all test panels.

Each laboratorian must decide whether the error rate is great enough to justify the additional expense involved in utilizing a second reader. The proficiency of the individuals involved can be tested by initiating a double reading program for a short trial period. We estimate that the second examination of each microdilution tray requires an average of 1.5 min, requiring approximately 30 to 45 min of technologist time and of laboratory assistant/clerk time each

day. As a result of this study, we have abandoned the precaution of double reading all test panels. Instead, our ongoing in-service training and quality control programs have been reinforced. Each day, five randomly selected test trays are examined by a second reader to monitor the performance of the different technologists responsible for reading the test results. When the two readers disagree by more than one dilution interval, the source of the problem is sought and corrected. Training of new technologists and student technologists is essential to obtain consistently uniform results, and that is accomplished by a double reading program. In such a program, timing is critical because the appearance of the growth patterns can change if more than 2 h elapse between the two readings.

LITERATURE CITED

1. **Barry, A. L.** 1979. A system for reporting quantitative antimicrobial susceptibility test results. Am. J. Clin. Pathol. **72**:864–868.
2. **Hoeprich, P. D., A. L. Barry, and G. D. Fay.** 1971. Synthetic medium for susceptibility testing, p. 494–497. Antimicrob. Agents and Chemother. 1970.
3. **Petralli, J., E. Russell, A. Kataoka, and T. C. Merigan.** 1970. On-line computer quality control of antibiotic-sensitivity testing. N. Engl. J. Med. **283**:735–738.
4. **Swenson, J. M., and C. Thornsberry.** 1978. Susceptibility tests for sulfamethoxazole-trimethoprim by a broth microdilution procedure. Curr. Microbiol. **1**:189–193.