

Evaluation of the Sensititre System for Quantitative Antimicrobial Drug Susceptibility Testing: a Collaborative Study

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This three-center collaborative study was conducted to evaluate samples of Sensititre antimicrobial microdilution panels (GIBCO/INVENEX). Sensititre minimum inhibitory concentrations of 27 bacterial isolates were compared with those obtained by a reference microdilution method. The Sensititre and microdilution minimum inhibitory concentrations were equivalent within ± 1 dilution in 87.6% of the comparable test results. Intralaboratory reproducibilities of the Sensititre and microdilution endpoints were equivalent with 80.4 and 82.4%, respectively, of on-scale endpoints in absolute agreement. Sensititre was more reproducible among laboratories, with nearly a 10% greater agreement of triplicate results. The Sensititre microdilution test as evaluated gave results which were essentially equivalent to those obtained with a standardized microdilution method.

Microdilution procedures for quantitative antimicrobial susceptibility testing have been in use for a number of years (3, 4). This study evaluates a commercial microdilution product, Sensititre. Sensititre antimicrobial susceptibility panels provide dried, stabilized antimicrobics in microdilution trays in appropriate concentrations for the determination of the minimal inhibitory concentration (MIC) of antimicrobial agents. The panels are manufactured by Seward Laboratory, London, England, and are distributed by GIBCO/INVENEX in the United States and Canada.

Philips et al. (5, 6) reported work done on an initial antimicrobial format developed by Seward Laboratory. This format has been altered to reflect current therapeutic practice in the United States. Sensititre plates are available in three formats: gram positive, gram negative, and urinary. Each Sensititre plate contains doubling dilutions of 11 different antimicrobial agents, and appropriate antimicrobial-free wells for growth and sterility controls. The salient feature of this product is the technology to pre-dose accurately the microdilution wells with stabilized antimicrobial solutions which are then dried, thereby producing a ready-prepared, disposable system. The stability of these antimicrobial drugs is said to be excellent when stored at room temperature for several months (5, 6).

A three-center collaborative study was planned to evaluate samples of Sensititre anti-

microbial microdilution panels. Sensititre MICs were compared with the MICs obtained with microdilution trays prepared in each of the collaborating laboratories by using the Dynatech MIC-2000 (Cooke Engineering Co., Alexandria, Va.) system. Microdilution reference MICs obtained with this system have been shown to correlate well with those obtained with the standardized World Health Organization International Collaborative Study reference broth dilution method (1, 2).

MATERIALS AND METHODS

Test strains. Twenty-seven bacterial isolates (Table 1) were tested by the two methods on 3 separate days in each of the three participating laboratories. These strains were selected to ensure the maximum number of MICs falling within the range of antimicrobial concentrations provided in the Sensititre panels. Only MIC endpoints one well removed from the extreme high and low concentrations for each antimicrobial can be considered to be on scale. The test organisms were further selected with the objective of having at least four on-scale endpoints for each antimicrobial agent and have these endpoints determined by four different species. For most antimicrobial drugs these objectives were met.

Three separate Sensititre antimicrobial panels were used in this study. Table 2 lists the antimicrobials and the range of drug concentrations tested for each of these panels. Currently available panels may vary somewhat from the prototypes used in this study. Reference microdilution panels duplicated the antimicrobial and drug concentration range of the Sensi-

titre panels. All panels were tested with all 27 organisms.

Microdilution tests. The reference microdilution test panels were prepared in each of the three participating laboratories with the Dynatech MIC-2000 system for filling and for inoculation as previously described (1). Endpoints were independently determined by two experienced technologists in each laboratory. If a disagreement in endpoint interpretation occurred, an independent determination was made by a third technologist.

To verify purity and final inoculum density, quantitative subcultures were made from the growth control well. The resulting colony counts were classified into four categories: $<5 \times 10^4$ colony-forming units (CFU) per ml; 5×10^4 to 1×10^5 CFU/ml; 1×10^5 to 5×10^5 CFU/ml; and $>5 \times 10^5$ CFU/ml.

Sensititre tests. GIBCO/INVENEX provided samples of the three antimicrobial panels bearing the

same lot numbers to each of the laboratories. Sensititre test panels were handled according to the instructions of the manufacturer. Each well of the Sensititre plates was rehydrated with 0.05 ml of Mueller-Hinton broth (Difco lot 631999) with a Microdrop 1 dispenser (Cooke Engineering no. 1-235-01). Colonies for testing were selected and inoculated into 0.5 ml of brain heart infusion broth and incubated for 4 to 6 h at 35°C. A sterile disposable-tip pipette was used to transfer 0.04 ml of the brain heart infusion broth culture to 40 ml of sterile distilled water containing 0.02% Polysorbate-80. After thorough mixing, the entire amount was poured into a sterile disposable inoculum tray. With the use of a separate sterile disposable plastic 96-prong inoculator (Cooke Engineering no. 5-999), approximately 0.005 ml of the inoculum was transferred to each well of the hydrated Sensititre panel. The final inoculum density was 5×10^4 to 5×10^5 CFU/ml.

Panels were sealed, incubated, read, and interpreted as with the microdilution method. Similarly, inoculum purity and density were verified as previously described.

TABLE 1. Bacterial isolates utilized in this study

Organism	No. tested
<i>Streptococcus faecalis</i>	3
<i>Streptococcus faecium</i>	1
<i>Streptococcus avium</i>	1
<i>Streptococcus durans</i>	1
<i>Staphylococcus aureus</i>	2
<i>Staphylococcus epidermidis</i>	4
<i>Escherichia coli</i>	2
<i>Proteus mirabilis</i>	2
<i>Providencia rettgeri</i>	1
<i>Providencia stuartii</i>	1
<i>Klebsiella pneumoniae</i>	4
<i>Enterobacter cloacae</i>	1
<i>Enterobacter aerogenes</i>	1
<i>Pseudomonas aeruginosa</i>	2
<i>Acinetobacter anitratus</i>	1

RESULTS

Accuracy. Each microdilution MIC was compared with each matching Sensititre MIC performed in parallel, and results were expressed as an MIC ratio (microdilution MIC/Sensititre MIC). If the MICs obtained by both methods were identical, the ratio was 1. If the microdilution method gave a larger MIC, the ratio was 2, 4, etc.; and if the Sensititre method gave larger MICs, the ratio was 0.5, 0.25, etc. Data submitted by all three participants are summarized in Table 3. Valid comparisons could not be made if one or both endpoints were either greater than the highest concentration of antimicrobial drug

TABLE 2. Sensititre antimicrobial test panel concentrations after the addition of 50 μ l of broth

Antimicrobial agent	Panel concn (μ g/ml)		
	Gram positive	Gram negative	Urine
Penicillin G	0.06-8		
Methicillin	0.125-16		
Ampicillin	0.125-16	0.25-32	1-128
Cephalothin	0.5-64	1-128	1-128
Gentamicin	0.125-16	0.125-16	0.5-64
Kanamycin	0.5-64	0.5-64	2-256
Erythromycin	0.25-32		
Chloramphenicol	0.25-32	0.5-64	
Clindamycin	0.125-16		
Tetracycline	0.125-16	0.25-32	0.5-64
Vancomycin	0.25-32		
Carbenicillin		4-512	4-512
Amikacin		0.25-32	
Tobramycin		0.125-16	
Colistin		0.125-16	1-128
Sulfisoxazole		0.5-64	2-256
Trimethoprim-sulfamethoxazole			0.25/4.75-32/608
Nalidixic Acid			1-128
Nitrofurantoin			2-256

TABLE 3. Comparison of microdilution and Sensititre MIC ratios

Testing laboratory ^a	No. of tests	MIC ratio (% of tests) ^b				
		≥4	2	1	0.5	≤0.25
Gram-positive panels						
Sacramento	278	2.5	18.0	51.8	22.3	5.4
Portland	305	4.9	16.1	35.4	40.0	3.6
Cleveland	294	4.1	11.9	33.0	37.8	13.3
Total	877	3.9	15.3	39.8	33.6	7.4
Gram-negative panels						
Sacramento	284	4.6	27.5	49.3	14.8	3.9
Portland	306	10.2	11.4	44.1	29.7	4.6
Cleveland	312	10.3	13.5	37.5	33.3	5.4
Total	902	8.5	17.2	43.5	26.3	4.8
Urine panels						
Sacramento	320	9.0	22.5	48.4	17.5	2.5
Portland	312	8.3	11.5	42.3	35.6	2.2
Cleveland	300	7.1	11.7	32.3	40.7	8.3
Total	932	8.2	15.3	41.2	31.0	4.2
All-panel Total	2,711	6.9	15.9	41.5	30.3	5.5

^a Matched pairs of tests were performed in three separate laboratories on 3 separate days (off-scale endpoints excluded).

^b Boldface figures are the most frequently observed MIC ratios

tested or equal to or less than the lowest concentration tested; these data were excluded from the summary. Of the 2,711 pairs of valid endpoints, 2,374 (87.6%) gave MIC ratios of 0.5, 1, or 2; i.e., the Sensititre MIC in each of these instances was found to be within one doubling dilution of the microdilution MIC.

Table 3 lists the distribution of MIC ratios obtained by the three laboratories and some differences between laboratories. With the gram-positive panels, tests performed in Portland and Cleveland showed a shift of the modal ratios (boldface figures) to 0.5. In the case of gram-negative panels, all three laboratories showed consistent modal MIC ratios of 1, and the urine panel tests performed in Cleveland again showed a shift in modal ratios to 0.5.

Table 4 lists the MIC ratios of all three laboratories by antimicrobial agent. Of the 19 antimicrobial agents tested on one or more of the panels, 14 had modal MIC ratios of 1. Exceptions were cephalothin, gentamicin, colistin, trimethoprim-sulfamethoxazole, and nitrofurantoin, which gave modal ratios of 0.5, 2, 2, 0.5, and 0.5, respectively. Although cephalothin was the only beta-lactam antibiotic with an MIC ratio less than 1, examination of the data showed a tendency toward lower ratios, with 10% of MIC ratios less than 0.5 in this category of antimicrobial drugs. On the other hand, the aminoglycoside antibiotics show a tendency toward higher MIC ratios (18% of MIC ratios greater than 2). Co-

listin showed the greatest variation, with 50% of the ratios being greater than 2.

Table 5 lists the data of all three laboratories by organism group and by major antimicrobial class. The previously noted shifts in the modal MIC ratio for beta-lactam and aminoglycoside antimicrobial drugs are most marked in the streptococcus group. With the staphylococcus group, the modal MIC ratio for the other antimicrobial class was shifted to 0.5. However, the beta-lactam and aminoglycoside groups tended to have lower MIC ratios as well. Enteric and nonenteric gram-negative bacilli showed good correlation, with modal MIC ratios of 1 and 91.8% and 90.1%, respectively, of ratios 2, 1, or 0.5.

Intralaboratory reproducibility of both the microdilution and Sensititre ratios was assessed by noting the degree of agreement of the three MICs obtained in each laboratory on 3 separate days. Similarly, interlaboratory reproducibility was measured by the agreement of the three MICs obtained in each laboratory on replicate testing days 1, 2, and 3. Each day was considered separately in the analysis. Complete agreement was obtained when all three MICs were identical. If two of the three MICs agreed and the third was different, agreement was considered to be 66.7%. There was 0% agreement when all three MICs were different.

Table 6 lists the intralaboratory reproducibility for the three laboratories; only on-scale end-

TABLE 4. Comparison of microdilution and Sensititre MIC ratios by antimicrobial agent^a

Antimicrobial agent	No. of tests	MIC ratio (% of tests) ^b				
		≥4	2	1	0.5	≤0.25
Methicillin	100		12.8	38.3^c	38.3	10.6
Penicillin G	47	1.0	10.0	42.0	35.0	12.0
Ampicillin	257	0.8	7.0	42.8	37.4	12.1
Cephalothin	277	1.1	5.5	35.6	49.5	8.4
Gentamicin	248	26.4	35.4	30.1	6.9	1.2
Kanamycin	240	13.0	27.3	45.5	11.8	2.5
Amikacin	76	2.6	30.3	53.9	9.2	3.9
Tobramycin	96	19.8	26.0	40.6	13.5	
Erythromycin	75	2.7	40.0	40.0^c	14.7	2.6
Chloramphenicol	235		4.3	43.2	45.7	6.8
Clindamycin	43		16.3	44.2	37.2	2.3
Tetracycline	264	1.2	10.3	51.1	36.3	1.1
Vancomycin	87		5.7	54.0	37.9	2.3
Carbenicillin	198	0.5	25.8	39.4	24.7	9.6
Colistin	96	50.0	26.0	14.6	5.2	4.2
Sulfisoxazole	91	8.8	18.7	46.2	23.1	3.3
Trimethoprim-sulfamethoxazole	33		6.1	42.4	45.5	6.1
Nalidixic Acid	95	2.2	8.4	49.5	36.8	3.2
Nitrofurantoin	153	0.7	5.2	39.2	49.7	5.3
Total	2,711	7.0	16.2	41.3	30.1	5.4

^a Matched pairs of tests were performed in three separate laboratories on 3 separate days (off-scale endpoints excluded).

^b Boldface figures are the most frequently observed ratios.

^c Median MIC ratio.

TABLE 5. Comparison of microdilution and Sensititre MIC ratios by antimicrobial group and organism group (off-scale endpoints excluded)

Organisms group/ antimicrobial group	No. of tests	MIC ratio (% of tests) ^a				
		≥4	2	1	0.5	≤0.25
Streptococcus						
β-Lactams	373	0.3	13.2	32.0	44.1	10.4
Aminoglycosides	263	39.8	32.8	21.0	5.0	1.5
Others	351	8.0	13.4	47.7	33.1	3.2
Total	987	11.5	18.5	34.7	29.8	5.5
Staphylococci						
β-Lactams	112	0.9	9.9	40.5	36.0	12.6
Aminoglycosides	19		5.6	44.4	44.4	5.6
Others	191	8.4	5.3	34.2	43.7	8.5
Total	322	5.3	6.9	37.0	41.1	9.7
Enterobacteriaceae						
β-Lactams	342	1.5	10.9	46.0	33.1	8.5
Aminoglycosides	284	4.2	31.4	49.8	12.4	2.1
Others	511	5.4	13.1	43.3	35.5	2.7
Total	1,137	3.9	17.0	45.8	29.9	4.3
Nonenteric gram-negative bacilli						
β-Lactams	53		5.7	47.2	32.1	15.1
Aminoglycosides	90	1.1	26.7	62.2	10.0	
Others	122	11.6	12.4	45.5	28.1	2.5
Total	265	5.7	15.9	51.5	22.7	4.2

^a Boldface figures are the most frequently observed MIC ratios.

points are presented. Overall, there was an 82.4% agreement of MICs for the microdilution method and 80.4% agreement for the Sensititre method.

The variation in agreement ranged from 77.6% (Cleveland, gram-negative panel) to 85.7% (Portland, gram-negative) for the microdilution method. With Sensititre the range of agreement was 75.4% (Cleveland, gram-negative panel) to 85.9% (Portland, gram-negative.)

Table 7 illustrates the results of the analysis of interlaboratory reproducibility of on-scale endpoints. There is a significant difference ($P < 0.001$) between the results obtained by the two test methods. Overall, 52.5% agreement was obtained with the microdilution method, and 64.9% was obtained with the Sensititre method. With these on-scale endpoints 86% of the discrepant MICs were \pm dilution interval from the majority MIC.

Inoculum density. The target inoculum density for these tests was 5×10^4 to 5×10^5 CFU/ml. Table 8 shows the distribution of colony counts in four ranges. Sensititre trays inoculated with a Cooke Engineering plastic disposable inoculator yielded 65.2% of the counts within the target range and 4.1% below and 30.8% above. Microdilution trays inoculated with the Dynatech MIC-2000 semiautomated inoculator yielded similar results with 62.5% of counts within the target range and 8.2% below and

29.3% above. Both methods tended to deliver inocula greater than that intended.

DISCUSSION

Accuracy and reproducibility (both intra-laboratory and interlaboratory) are important in evaluating any new antimicrobial susceptibility testing method. In this study accuracy was assessed by direct comparison of Sensititre MICs with those obtained by a reference microdilution procedure. From a practical point of view, most techniques that involve serial twofold dilutions are considered satisfactorily controlled if the results of independent tests vary no more than ± 1 dilution. The Sensititre and microdilution MICs were equivalent within this range in 87.6% of the comparable test results. In some instances of individual antimicrobial drugs, at times associated with certain test organisms, a tendency toward higher or lower MIC as determined by Sensititre was observed. This was most marked with the beta-lactam antibiotics showing higher MICs and aminoglycoside antibiotics showing lower MICs. In both instances most of MIC endpoints contributing to the bias were produced by the strains of streptococci used in this study. Although aminoglycosides were tested against these streptococcal strains, including various enterococci, in a clinical situation these drugs could not be used for therapy. Strain selection was based on the technical grounds of

TABLE 6. *Intralaboratory reproducibility of microdilution and Sensititre MIC endpoints within each of three laboratories performing replicate tests on each of 3 separate days (off-scale endpoints excluded)*

Panel type	Laboratory	Method ^a	No. of tests	% Agreement ^b
Gram positive	Sacramento	MR	312	81.7
		ST	276	80.4
	Portland	MR	315	85.7
		ST	333	85.9
	Cleveland	MR	333	79.3
		ST	312	76.3
Gram negative	Sacramento	MR	288	83.7
		ST	285	78.6
	Portland	MR	327	84.7
		ST	327	82.0
	Cleveland	MR	330	77.6
		ST	345	75.4
Urinary	Sacramento	MR	315	85.4
		ST	315	80.0
	Portland	MR	309	84.8
		ST	330	85.2
	Cleveland	MR	327	79.2
		ST	354	79.9
All	All	MR	2,856	82.4
		ST	2,877	80.4

^a MR, Microdilution; ST, Sensititre.

^b Percent agreement with most frequently observed endpoint in each set of three replicate tests in each laboratory; (e.g., 16, 16, 16 = 3/3 = 100%; 16, 16, 8 = 2/3 = 66.7%; 32, 16, 8 = 0/3 = 0%).

TABLE 7. *Interlaboratory reproducibility of microdilution and Sensititre*

Panel type	Method ^b	No. of tests	% Agreement ^a
Gram positive	MR	813	50.4
	ST	873	64.5
Gram negative	MR	801	53.7
	ST	843	65.6
Urine	MR	771	53.3
	ST	867	64.7
All	MR	2,382	52.5
	ST	2,583	64.9

^a MIC end points were determined among three laboratories performing replicate tests (off-scale end-points excluded).

^b MR, Microdilution; ST, Sensititre.

^c Percent agreement with most frequently observed endpoint in each of three replicate tests in three separate laboratories on each of 3 days (e.g., 16, 16, 16 = 3/3 = 100%; 16, 16, 8 = 2/3 = 66.7%; 32, 16, 8 = 0/3 = 0%).

TABLE 8. *Comparison of inoculum densities obtained with the Sensititre disposable plastic inoculator and Dynatech automated inoculator*

Inoculum density (CFU/ml)	Sensititre inoculator		Dynatech inoculator	
	No.	%	No.	%
<5 × 10 ⁴	17	4.1	34	8.2
5 × 10 ⁴ –1 × 10 ⁵	37	8.9	63	15.1
1 × 10 ⁵ –5 × 10 ⁵	234	56.3	197	47.4
>5 × 10 ⁵	128	30.8	122	29.3

providing the maximum number of on-scale end-points for valid comparisons. Colistin proved to be the least accurate of all the antimicrobial drugs tested, with 50% of the MIC ratios equal to or greater than 4. Colistin and polymixin B have been notoriously difficult to evaluate in microdilution systems, with skipped wells a frequent occurrence. Based on our experience these antimicrobial agents should not be tested by this procedure. Variations in MIC results observed with sulfisoxazole are most likely related to the subjective interpretation of the endpoints. End-points are not sharp and considerable "tailing", i.e., gradually decreasing turbidity, is observed. This leads to decreased reproducibility of end-points both within and among laboratories. Nearly 9% of the MIC ratios were equal to or greater than 4.

Intralaboratory reproducibility of Sensititre and microdilution endpoints were equivalent with 80.4 and 82.4%, respectively, of on-scale endpoints in absolute agreement.

Although intralaboratory reproducibility is considered equivalent, Sensititre is more reproducible among laboratories, with nearly a 10% greater agreement of triplicate test results. This

can be explained by the fact that each laboratory was provided with identical materials for performing Sensititre tests, and all panels were from the same lot. In the microdilution reference method, although each investigator was supplied with the same lots of assayed antimicrobial standard materials, each laboratory individually weighed, dissolved, diluted, and dispensed these materials. Consequently, a number of additional variables were introduced in the production of microdilution trays as compared with Sensititre. These observations support the greater degree of standardization that can be obtained when susceptibility testing materials are prepared in a central laboratory in large batches, with a greater degree of control than might be obtained in a clinical laboratory.

The density of inoculum delivered by the two systems of inoculation gave equivalent results, although both methods tended to deliver inocula that resulted in colony counts greater than expected. Simple adjustment of the initial dilution steps involved should result in a final inoculum with a greater percentage of counts in the 5 × 10⁴ to 5 × 10⁵ range.

We conclude that the Sensititre microdilution test gives results which are essentially equivalent to those obtained with a standardized microdilution method. Furthermore, interlaboratory reproducibility of a single lot of Sensititre panels was greater than that of individual laboratory-prepared microdilution panels. Microdilution tests of aminoglycosides against enterococci were unreliable, especially when tested in 50- μ l volumes. Except for tests with sulfisoxazole and colistin, the Sensititre trays were satisfactory.

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