

Comparison of Two Automated Instrument Systems for Rapid Susceptibility Testing of Gram-Negative Bacilli

J. E. JOHNSON,^{1,2*} J. H. JORGENSEN,² S. A. CRAWFORD,² J. S. REDDING,² AND R. C. PRUNEDA³

Laboratory Service, Audie Murphy Veterans Administration Hospital¹; Department of Pathology, The University of Texas Health Science Center;^{2*} and The Severance Reference Laboratory,³ San Antonio, Texas 78284

Received 23 June 1983/Accepted 20 September 1983

The Vitek AutoMicrobic System with GSC-plus cards and the Abbott MS-2 system were tested in parallel and the results were compared directly with those of a reference microdilution minimal inhibitory concentration (MIC) procedure on a group of 262 clinical isolates of the family *Enterobacteriaceae* and of *Pseudomonas aeruginosa*. Results of both systems were compared with the reference MIC for category agreement, and in addition, the Vitek MICs were compared with those obtained by the reference procedure. The Vitek system provided an essential category correlation of 89.4% for enteric bacteria and 97.0% for *P. aeruginosa*. Vitek MICs agreed within 1 twofold dilutional increment for 86.3% of the enteric bacteria tested and for 96.2% of the *P. aeruginosa* isolates. The Abbott MS-2 essential category agreement was 92.0% for enteric bacteria and 92.4% for *P. aeruginosa*. If only aminoglycosides or carbenicillin were considered for *P. aeruginosa* isolates, the essential category agreement was 92.5% for the Vitek and 93.3% for the MS-2. The majority of MS-2 category errors (13 of 19) with *P. aeruginosa* involved gentamicin results on isolates whose reference MICs were 8 µg/ml and whose MS-2 results were susceptible (MIC ≤ 4 µg/ml). Retesting of the *P. aeruginosa* isolates in calcium-supplemented MS-2 broth increased the essential agreement for the aminoglycosides to 97.5%.

Antimicrobial susceptibility testing has traditionally been performed by the manual Bauer-Kirby disk diffusion method (3), yielding a category result (susceptible, intermediate, or resistant), or more recently by a dilution procedure (4, 11), which provides a quantitative result, the minimal inhibitory concentration (MIC). In recent years, a number of semiautomated and automated instruments have become available for performing bacterial identification and susceptibility tests. Certain of these, the Autobac (General Diagnostics, Plainview, N.J.), the Abbott MS-2 (Abbott Laboratories, Dallas, Tex.), and the Vitek AMS (Vitek Systems, Inc., Hazelwood, Mo.), provide rapid, same-day results (1, 14, 16).

The Vitek AMS is a computerized system capable of enumerating and identifying a variety of bacteria and yeasts (1). Susceptibility testing of *Enterobacteriaceae* and of *Pseudomonas aeruginosa* was previously performed with the general susceptibility card (GSC), in which category results (susceptible, intermediate, or resistant) were given. More recently, Vitek has marketed the GSC-plus card designed to provide rapid MIC reporting. The system also categorizes results according to the proposed catego-

ries of the National Committee for Clinical Laboratory Standards (NCCLS), very susceptible, moderately susceptible, and resistant (12).

The Abbott MS-2 utilizes a modified category report system of susceptible (≤ a defined MIC), intermediate, and resistant (≥ a defined MIC). If a result is in the intermediate range, a calculated MIC is given (14).

In an effort to determine the accuracy of these systems for susceptibility testing of members of the *Enterobacteriaceae* and *P. aeruginosa* in a clinical laboratory setting, the Vitek AMS and the Abbott MS-2 were tested in parallel, and the results were compared with a reference microdilution MIC procedure.

MATERIALS AND METHODS

Organisms. A total of 262 isolates of the family *Enterobacteriaceae* and of *P. aeruginosa* were included in the study group. They consisted primarily of recent clinical isolates from the Audie Murphy Veterans Administration Hospital Microbiology Laboratory and selected stock cultures of antibiotic-resistant isolates which had been collected previously. To verify the adequacy of the reference microdilution procedure, each set of tests included the control organisms *Escherichia coli* ATCC 25923, *P. aeruginosa* 27853, and *Streptococcus faecalis* ATCC 29212. Each set of

AMS and MS-2 tests included simultaneous testing of the same *E. coli* and *P. aeruginosa* control strains to verify proper performance of the instruments.

Reference microdilution MIC procedure. As a reference procedure, microdilution trays were prepared with a Dynatech MIC 2000 according to the NCCLS recommendations (12). The antibiotics used and their concentration ranges (in micrograms per milliliter) were as follows: amikacin, 1 to 32; ampicillin, 0.25 to 8; chloramphenicol, 1 to 32; cephalothin, 1 to 32; cefamandole, 1 to 32; cefoxitin, 1 to 32; gentamicin, 0.5 to 16; tobramycin, 0.5 to 16; and tetracycline, 0.5 to 16. Carbenicillin (8 to 256 $\mu\text{g/ml}$) was tested only against *P. aeruginosa*. The above antibiotics were compared for all three systems tested. The Mueller-Hinton broth used in the microdilution reference procedure was supplemented to a final concentration of 50 mg of calcium per liter and 25 mg of magnesium per liter (12). A single lot of microdilution trays was prepared for this study and stored at -70°C .

Vitek AMS. The GSC-plus card is a sealed plastic template containing two to four concentrations of 10 different antibiotics. Suspensions of fresh bacterial isolates from 18 to 24 h of growth on sheep blood agar were prepared in 0.45% saline to a turbidity equivalent to a no. 1 McFarland standard and were transferred to the cards according to the directions of the manufacturer. The inoculated cards (lot no. 522C) were incubated and analyzed with a Vitek AMS 120 instrument. Upon completion of a susceptibility test, MIC results were automatically printed, along with an interpretation of very susceptible, moderately susceptible, or resistant. However, with each test it was necessary to locate the proper result for a given isolate by examining five different response panels listed according to isolate identity as follows: *E. coli*, *Proteus mirabilis*, *Proteus* spp., *Klebsiella* spp., and others). Vitek results were evaluated by agreement within 1 twofold dilutional increment of the reference microdilution system results and by agreement with the NCCLS category indicated by the instrument as compared with that obtained by the reference procedure.

Abbott MS-2. An Abbott MS-2 clinical system was employed with standard MS-2 transfer cartridges, elution disks, and test medium (Oxoid Sensi-Test Broth). Inocula for the MS-2 were prepared from 18 to 24 h-old growth obtained on sheep blood agar. A suspension visually equivalent to a 0.5 McFarland opacity standard was prepared for each isolate in 0.85% sterile saline. A sample of 200 μl of the suspension was transferred to a MS-2 cartridge, which contained growth medium and selected elution disks. Each cartridge was then inserted into an instrument-defined position in one of the analysis modules of the MS-2. All subsequent operations were completed by the MS-2. Results included a statement of susceptible, intermediate, or resistant and also included a correlating MIC range if isolates were susceptible (the content of the elution disk or less) or resistant (three times the content of the elution disk or more). If an intermediate response was obtained, a programatically calculated MIC was printed. Because the MS-2 does not purport to generate a full-range MIC, the accuracy of MS-2 results was evaluated by comparison of the MIC correlate (based on the content of the elution disk) of the MS-2 and the reference MIC for that isolate. Thus, for example, if the MS-2 printed a result for ampicillin

of "susceptible, MIC $\leq 10 \mu\text{g/ml}$ " and the reference MIC was 8 $\mu\text{g/ml}$, it was considered as full agreement. Likewise, a response of "resistant, MIC $\geq 30 \mu\text{g/ml}$ " when the reference MIC was 32 $\mu\text{g/ml}$ was considered correct. If, on the other hand, the MS-2 result was "susceptible, MIC $\leq 10 \mu\text{g/ml}$ " and the reference MIC was 16 $\mu\text{g/ml}$, it was classified as essential agreement or a minor error since the intermediate range of the MS-2 should include an MIC of 16 $\mu\text{g/ml}$ (the MS-2 intermediate range was 11 to 29 $\mu\text{g/ml}$ between one and three times the elution disk content).

Categorization of results. The terms used in interpreting the results of the study are defined as follows. Very major discrepancy: Susceptible by the instrument method when resistant by the reference method (includes very susceptible or moderately susceptible categories for Vitek). Major discrepancy: Resistant by the instrument method when susceptible by the reference method. Minor discrepancy: (i) Intermediate by the MS-2 when susceptible or resistant by the reference method or intermediate by the reference method when susceptible or resistant by MS-2; or (ii) Very susceptible by the Vitek when moderately susceptible by the reference method or very susceptible by the reference method when moderately susceptible by the Vitek. Complete agreement: Very major, major, and minor discrepancies were considered as errors. Essential agreement: Only very major and major discrepancies were considered as errors.

RESULTS

A comparison of category results for *Enterobacteriaceae* by the Vitek AMS GSC-plus card with results by the microdilution reference method is shown in Table 1. Complete agreement was noted in 75% of 1,998 tests, with 89.4% of the tests demonstrating essential agreement (minor errors excluded). Complete agreement varied from 48.6% for cefoxitin to 96% for amikacin. *P. mirabilis*, *Providencia* spp., and *Serratia* spp. showed the lowest overall correlations among the organisms tested.

Table 2 shows the category results for enteric organisms tested by the MS-2 and compares those results with results by the microdilution procedure. Complete agreement was observed in 92% of all tests, and essential agreement was observed in 96%. Complete agreement varied from 85% for chloramphenicol to 98.7% for amikacin, whereas essential agreement varied from 89% for cephalothin to 99.6% for gentamicin. *Citrobacter freundii*, *Providencia rettgeri*, and *Serratia* spp. showed the lowest correlations among the organisms tested.

Tables 3 and 4 demonstrate the category correlations for the Vitek and Abbott systems against the microdilution procedure for *P. aeruginosa*. Complete agreement was observed in 95.5% of tests with the Vitek and 92.4% of tests with the MS-2. Essential agreement was 97.2% for the Vitek and 97.5% for the MS-2. If only the aminoglycosides and carbenicillin are compared, the Vitek demonstrated 88% complete

TABLE 1. Agreement between category results for *Enterobacteriaceae* in tests with the Vitek GSC-plus and the NCCLS reference procedure

Organism	Strains tested	No. of discrepancies ^a												Complete Agreement ^b	
		Amika-cin	Ampi-cillin	Cepha-lothin	Cefoxi-tin	Cefaman-dole	Chloram-phenicol	Genamycin	Tobramy-cin	Tetracy-cline	No. agreeing/ no. of tests	%	No. agreeing/ no. of tests	%	
<i>Citrobacter diversus</i>	9	0,0,0	0,0,0	0,0,1	0,1,4	0,0,1	0,2,0	0,0,1	0,0,6	0,0,4	64/81	79.0	78/81	96.4	
<i>Citrobacter freundii</i>	14	0,0,0	0,1,0	0,0,0	4,1,0	0,2,1	2,1,1	0,1,2	0,1,5	1,0,4	99/126	78.5	112/126	88.9	
<i>Enterobacter aerogenes</i>	12	0,0,2	1,0,0	1,0,1	2,0,1	0,0,2	0,4,0	0,0,4	0,0,9	0,0,0	81/108	75.0	100/108	92.5	
<i>Enterobacter agglomerans</i>	6	0,0,0	0,0,0	1,0,0	1,0,2	0,1,1	0,3,0	0,0,1	0,0,2	0,0,1	41/54	76.0	47/54	87.0	
<i>Enterobacter cloacae</i>	19	0,0,1	1,1,0	0,0,1	0,0,0	0,3,5	0,0,1	0,0,5	0,0,12	2,0,1	138/171	80.7	164/171	96.0	
<i>Escherichia coli</i>	40	0,0,6	1,0,4	0,2,5	1,1,16	0,0,3	2,1,4	0,1,13	0,3,25	1,0,3	281/360	78.0	347/360	96.4	
<i>Klebsiella oxytoca</i>	2	0,0,0	0,0,0	1,0,0	0,0,1	0,0,1	0,0,0	0,1,0	0,1,0	0,0,1	12/18	66.7	15/18	83.3	
<i>Klebsiella pneumoniae</i>	43	0,4,3	1,0,0	4,1,7	2,2,10	4,3,3	0,7,1	0,1,0	0,1,0	1,1,4	299/387	77.2	352/387	91.0	
<i>Morganella morganii</i>	12	0,0,5	0,0,0	0,0,0	0,0,0	0,7,1	0,0,0	0,0,3	0,0,12	0,0,3	77/108	71.2	101/108	93.5	
<i>Proteus mirabilis</i>	29	0,3,10	0,3,5	0,0,8	3,0,10	1,1,4	1,1,0	0,4,8	0,4,14	0,0,1	180/261	69.0	240/261	91.9	
<i>Proteus vulgaris</i>	4	0,0,0	0,0,0	0,0,0	0,0,1	0,1,0	0,1,0	0,0,0	0,0,1	0,0,0	32/36	88.9	34/36	94.5	
<i>Providencia rettgeri</i>	6	0,0,0	0,1,0	0,0,2	0,1,4	0,2,0	0,0,0	0,2,0	0,2,1	0,0,0	39/54	72.3	47/54	87.0	
<i>Providencia stuartii</i>	6	0,0,1	0,0,0	0,0,0	0,2,3	0,5,0	0,0,1	0,2,1	0,2,1	0,0,0	36/54	66.7	47/54	87.0	
<i>Salmonella</i> spp.	1	0,0,0	0,0,0	0,0,1	0,0,0	0,0,0	0,0,0	0,0,1	0,0,1	0,0,0	6/9	66.7	9/9	100	
<i>Serratia</i> spp.	19	0,2,2	0,0,0	1,1,0	3,3,0	0,2,0	3,4,0	0,1,5	0,5,1	8,0,1	129/171	75.5	138/171	80.7	
Complete agreement		82.5%	91.5%	82.9%	64.4%	75.7%	82.0%	68.4%	48.6%	83.4%					
Essential agreement		96.0%	95.5%	94.6%	76.6%	85.6%	85.6%	90.1%	86.0%	93.6%					

^a Values show very major, major, and minor discrepancies, in that order. See text for explanation of categories.
^b For explanation of terms, see text. Overall results: Complete agreement, 75.0% (1,498 out of 1,998); essential agreement, 89.4% (1,784 out of 1,998).

TABLE 2. Agreement between category results for *Enterobacteriaceae* in tests with the MS-2 and the NCCLS reference procedure

Organism	Strains tested	No. of discrepancies ^a											Agreement ^b		
		Amikacin	Ampicillin	Cephathioin	Cefoxitin	Cefamandole	Chloramphenicol	Gentamicin	Tobramycin	Tetracycline	Complete	Essential			
		No. of tests	%	No. of tests	%	No. of tests	%	No. of tests	%	No. of tests	%	No. of tests	%		
<i>Citrobacter diversus</i>	9	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	81/81	100	81/81	100
<i>Citrobacter freundii</i>	14	0,0,0	0,0,1	5,0,1	1,0,2	2,0,0	0,0,0	0,0,0	0,0,0	0,0,1	0,0,1	110/126	87.4	118/126	93.7
<i>Enterobacter aerogenes</i>	12	0,0,0	0,0,1	1,0,2	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	104/108	96.3	107/108	99.2
<i>Enterobacter agglomerans</i>	6	0,0,0	0,0,1	0,0,0	0,0,0	0,2,0	0,0,1	0,0,0	0,0,0	0,0,0	0,0,0	50/54	92.6	54/54	100
<i>Enterobacter cloacae</i>	19	0,0,0	0,2,1	1,1,0	1,0,1	1,0,0	1,0,6	1,0,2	0,0,0	2,0,0	2,0,0	154/171	90.0	163/171	95.8
<i>Escherichia coli</i>	40	0,1,0	1,3,0	0,1,0	0,0,1	0,1,0	1,0,2	0,0,1	0,0,1	1,0,0	1,0,0	345/360	95.8	351/360	97.5
<i>Klebsiella oxytoca</i>	2	0,0,0	0,0,0	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	17/18	94.4	17/18	94.4
<i>Klebsiella pneumoniae</i>	42	0,1,0	1,0,1	9,2,0	0,0,0	7,0,1	1,0,1	1,0,2	1,0,2	1,0,1	1,0,1	354/387	91.5	362/387	93.6
<i>Morganella morganii</i>	12	0,0,0	0,0,0	0,0,0	0,0,1	0,4,1	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	102/108	94.4	104/108	96.3
<i>Proteus mirabilis</i>	29	0,0,1	0,5,1	1,0,1	1,0,1	1,1,4	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	243/261	93.1	251/261	96.4
<i>Proteus vulgaris</i>	4	0,0,0	0,0,0	0,0,0	0,0,0	0,0,1	0,0,3	0,0,0	0,0,0	0,0,0	0,0,0	32/36	88.9	36/36	100
<i>Providencia rettgeri</i>	6	0,0,0	0,0,3	0,0,0	0,0,0	0,0,2	0,0,4	0,0,0	0,0,0	0,0,0	0,0,0	45/54	83.3	54/54	100
<i>Providencia stuartii</i>	6	0,0,0	1,0,0	0,0,0	0,0,0	0,1,1	0,0,2	0,0,0	0,0,0	0,0,0	0,0,0	49/54	90.7	52/54	96.4
<i>Salmonella</i> spp.	1	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	9/9	100	9/9	100
<i>Serratia</i> spp.	19	0,0,0	0,0,0	2,0,0	2,0,3	1,0,0	0,0,7	0,0,2	1,0,0	2,0,6	2,0,6	145/171	84.8	163/171	95.8
Complete agreement		98.7%	90.0%	87.4%	93.7%	86.0%	85.0%	97.4%	96.9%	93.7%	93.7%				
Essential agreement		99.2%	94.2%	89.0%	97.8%	90.5%	98.2%	99.6%	98.6%	97.4%	97.4%				

^a Values show very major, major, and minor discrepancies, in that order. See text for explanation of categories.

^b For explanation of terms, see text. Overall results: Complete agreement, 92.0% (1,831 out of 1,989); essential agreement, 96.0% (1,910 out of 1,989).

TABLE 3. Correlation between category results for *P. aeruginosa* in tests with the Vitek GCS-plus and the NCCLS reference procedure^a

Antibiotic	No. of discrepancies			Agreement (%)	
	Very major	Major	Minor	Complete	Essential
Amikacin	0	4	3	82.5	90
Ampicillin	0	0	0	100	100
Cephalothin	0	0	0	100	100
Cefoxitin	0	0	0	100	100
Cefamandole	0	0	0	100	100
Chloramphenicol	0	0	0	100	100
Gentamicin	0	2	0	95	95
Tobramycin	0	4	0	90	90
Tetracycline	0	0	0	100	100
Carbenicillin	0	2	4	85	95
Total				95.5	97.2

^a Categories of discrepancy and agreement are explained in the text.

agreement and 92.5% essential agreement, whereas the MS-2 dropped to 81.8% complete agreement and 93.3% essential agreement.

A summary of the antibiotic correlation by MIC for enteric organisms comparing the Vitek and microdilution methods is shown in Table 5. Agreement within 1 concentration increment was seen with 86.3% of tests. The highest numbers of discrepancies occurred with cefoxitin (58 of 222) and cefamandole (38 of 222). Figure 1 also demonstrates the MIC correlation for members of the family *Enterobacteriaceae*. Of 1,998 tests, 62% were in complete agreement, and 86.3% were within 1 dilution. The MIC results for *P. aeruginosa* (Fig. 2) include only data for the aminoglycosides and carbenicillin since the other antibiotics tested agreed 100%. There was 51.3% complete agreement, and 90.7% of the tests agreed within 1 dilution.

DISCUSSION

The past several years have heralded a trend away from traditional Bauer-Kirby disk diffusion tests in favor of either routine performance of full-range MIC tests (8, 11) or rapid, same-day quantitative or qualitative testing by an instrument method (8). Although individual preferences may dictate whether MIC methods with traditional incubation times (overnight) or rapid automated methods with same-day results are chosen, the accuracy of the results should not be allowed to suffer significantly. Thus, in choosing any alternative method, it is reasonable to expect results comparable to those of established traditional susceptibility test methods.

The Vitek AMS with GSC-plus cards for rapid quantitative susceptibility testing of *Enterobacteriaceae* gave results which were considerably

lower (<90%) than expected for both the MIC and category comparisons. Figure 1 demonstrates that the MIC discrepancies tended to be 1 twofold dilutional increment higher than the reference method results. The majority of category errors for the aminoglycosides were minor ones in which the reference MIC was 0.5 µg/ml (very susceptible) and the Vitek MIC was 1.0 µg/ml (moderately susceptible). Over 90% of the major errors for gentamicin and tobramycin occurred when the reference MIC was 4 µg/ml and the Vitek MIC was >4 µg/ml. The other large group of discrepancies was noted with the cephalosporin group of antibiotics when the reference MIC was 16 µg/ml and the Vitek MIC was >16 µg/ml or vice-versa. According to the NCCLS proposed interpretive categories, very susceptible, moderately susceptible, and resistant (12), the usual definition of very major, major, and minor discrepancies had to be modified for this study as mentioned above under categorization of results. There are no firmly established guidelines for accepting these results instead of the values of ≥90% for complete agreement and ≥95% for essential agreement recommended for the categories of susceptible, intermediate, and resistant (15). For that reason, category results were reported primarily as essential agreement, excluding the minor discrepancies (very susceptible versus moderately susceptible). Still, only 89% essential agreement and 86.3% correlation of MICs within 1 dilution for the enteric organisms were obtained with the Vitek. It is generally believed that dilution tests should have 95% agreement within 1 dilution (5). Other studies have indicated that the inoculum size may have a marked effect on rapid susceptibility test results (6, 10). Because the Vitek

TABLE 4. Correlation between category results for *P. aeruginosa* in tests with the MS-2 and the NCCLS reference procedure^a

Antibiotic	No. of discrepancies			Agreement (%)	
	Very major	Major	Minor	Complete	Essential
Amikacin	0	0	2	95.2	100
Ampicillin	0	0	0	100	100
Cephalothin	0	0	0	100	100
Cefoxitin	0	0	0	100	100
Cefamandole	0	0	0	100	100
Chloramphenicol	0	0	0	100	100
Gentamicin	6	0	13	54	85.4
Tobramycin	2	0	2	90.3	95.2
Tetracycline	0	0	0	100	100
Carbenicillin	0	3	3	85.4	92.7
Total				92.4	97.5

^a Categories of discrepancy and agreement are explained in the text.

TABLE 5. Correlation between Vitek GSC-plus MICs and reference dilution MICs^a

Organism	Strains tested	No. of discrepancies (tests with difference of over 1 dilution)										Tetracycline	No. of discrepancies/no. of tests	Organism correlation (%) ^b
		Amikacin	Ampicillin	Ceph- lothin	Cefoxitin	Cefam- dole	Chloram- phenicol	Gentami- cin	Tobramy- cin					
<i>Citrobacter diversus</i>	9				9	2	2						13/81	84.0
<i>Citrobacter freundii</i>	14		1	2	5	3	2						16/126	87.4
<i>Enterobacter aerogenes</i>	12	1	2	2	4	1		1					12/108	89.6
<i>Enterobacter agglomerans</i>	6				3	1	2		1				7/54	87.0
<i>Enterobacter cloacae</i>	19	1	3	1	4	4	2		2			3	16/171	90.6
<i>Escherichia coli</i>	40	5	7	7	11	3	1		7			9	50/360	86.1
<i>Klebsiella oxytoca</i>	2			1		1							2/18	88.9
<i>Klebsiella pneumoniae</i>	43	6	4	5	4	7	6		3	2	1		38/387	90.2
<i>Morganella morganii</i>	12	3				4	1		2	3			13/108	88.0
<i>Proteus mirabilis</i>	29	13	7	8	13	5	1		6	6			59/261	77.4
<i>Proteus vulgaris</i>	4					1	1						2/36	94.4
<i>Providencia rettgeri</i>	6	1	1	1	3	2			1	1			9/54	83.4
<i>Providencia stuartii</i>	6	2			4	4	1		1	1			13/54	75.9
<i>Salmonella</i> spp.	1		1	1									2/9	77.8
<i>Serratia</i> spp.	19	3			2				5	4	7		21/171	87.7
Antibiotic correlation ^b		84.3%	88.3%	87.4%	74.0%	82.9%	91.4%	87.9%	87.0%	94.1%				

^a Overall correlation, 86.3% (1,725 out of 1,998 tests).^b Percentage of agreement within 1 dilution.

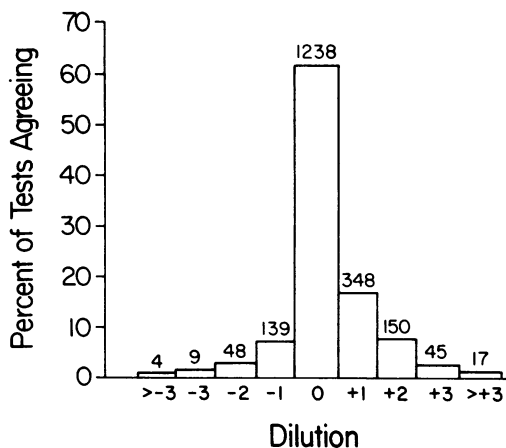


FIG. 1. Agreement by dilution between Vitek GSC-plus and a reference microdilution procedure for enteric organisms. Numbers shown at the top of each bar are the number of antibiotic tests that agreed. A total of 1,998 antibiotic tests were compared, of which 62% completely agreed and 86.3% of tests agreed within 1 dilution.

MICs were generally higher than the reference MICs, the possibility of the inocula being too large was considered. The Vitek GSC-plus package insert states in bold type that a density equal to a no. 1 McFarland standard must be used. However, a personal communication from Vitek Systems, Inc. stated that the inoculum should actually be lighter (approaching a 0.5 McFarland standard). To examine this possibility, a second group of 100 organisms was tested in the AMS only at two different inoculum densities, a 0.5 McFarland suspension and a no. 1 McFarland suspension. Results of susceptibility tests at both inoculum densities yielded the same category result (essential agreement) in >92% of tests and were within 1 twofold dilutional increment in 90% of the tests. Moreover, the central tendency of results with the 0.5 McFarland suspension inoculum was not lower than the no. 1 McFarland inoculum tests. Thus, inoculum density alone cannot explain a lack of agreement with the reference microdilution MICs. Moreover, MICs for the *E. coli* and *P. aeruginosa* control strains were always within 1 dilutional increment of their expected values at either inoculum density.

In this study, the AMS identification cards (EBC-plus) were not used in conjunction with the susceptibility cards (GSC-plus). This resulted in five potential susceptibility results being listed for each isolate (*E. coli*, *P. mirabilis*, *Proteus* spp., *Klebsiella* spp., and others), as mentioned previously. Based on the organism identification, the MIC of a given antibiotic would thus vary according to species. By mating

the identification and susceptibility test cards for a given isolate (by simultaneously inoculating and placing them into the same incubator tray), an identification would be indicated by the AMS with a single specific susceptibility profile. The use of mated cards would have simplified interpretation of results in this study and could decrease possible transcription errors in a clinical setting.

In a recent study by Hansen and Freedy (7), susceptibility test results produced by a variety of systems, including the Vitek with the older GSC card and the Abbott MS-2, were compared with the proposed NCCLS reference microdilution procedure. Although both of these systems yielded category results (susceptible, intermediate, or resistant), algorithms were developed to classify results according to MIC breakpoint criteria. Only major and very major discrepancies were considered in this analysis. With this type of classification, overall agreement (for both *Enterobacteriaceae* and *P. aeruginosa*) for Vitek was 84% when compared with reference microdilution MICs and 95% when compared with Bauer-Kirby disk diffusion results. With the GSC-plus card designed by Vitek to produce MICs, our overall agreement for both groups of organisms (enteric bacteria and *P. aeruginosa*) compared with reference MICs was 90.5%. Our data for both organism groups with the MS-2 yielded an overall (essential) agreement of 96.4%, very close to the 97% overall agreement noted by Hansen and Freedy with disk diffusion results as a comparison (7). However, only 87% agreement with MS-2 and microdilution MICs

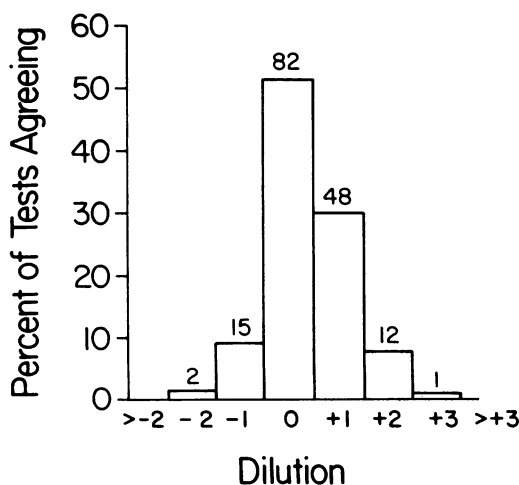


FIG. 2. Agreement by dilution between Vitek GSC-plus and a reference microdilution procedure for *P. aeruginosa*. Numbers shown at the top of each bar are the number of antibiotic tests that agreed. A total of 160 antibiotic tests were compared, of which 51.3% completely agreed and 89.7% agreed within 1 dilution.

were noted by Hansen and Freedy (7). Other studies comparing MS-2 results with disk diffusion or microdilution results (2, 14) and comparing MS-2 and AMS results with disk diffusion testing (9) have demonstrated very similar levels of agreement with the reference procedures.

Recently, the NCCLS has modified the proposed categories of very susceptible, moderately susceptible, moderately resistant, and very resistant to susceptible, moderately susceptible, resistant and conditionally susceptible (13). The MIC category breakpoints for individual drugs have likewise been revised. Although Vitek has modified its reporting system to include a result of susceptible, intermediate, or resistant as well as an MIC when using the GSC-plus card, still further studies are needed to determine what effect these changes may have on the accuracy of the Vitek System with the GSC-plus cards.

The Abbott MS-2 system gave results for *Enterobacteriaceae* which most clinical microbiologists would consider acceptable ($\geq 90\%$ complete agreement, $\geq 95\%$ essential agreement) (15). However, with *P. aeruginosa*, a lower correlation was noted with gentamicin (54% complete agreement, 85.4% essential agreement). Out of 19 discrepancies, 13 occurred in which reference MICs were 8 $\mu\text{g}/\text{ml}$ and the MS-2 results were susceptible ($\leq 4 \mu\text{g}/\text{ml}$). In an effort to correct this problem, the divalent cation content of the MS-2 broth was determined chemically (atomic adsorption spectrophotometry) and found to contain approximately the currently recommended (12) level of magnesium (28 mg/liter, versus 25 ml/liter recommended) and approximately one-half the suggested calcium content (21 mg/liter, versus 50 mg/liter recommended). The MS-2 broth was then supplemented up to the suggested 50 mg/liter level of calcium by aseptic addition of a predetermined volume of ice-cold filter-sterilized $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ into ice-cold, previously autoclaved MS-2 broth. All *P. aeruginosa* isolates which had produced discrepant results were then retested with the calcium-supplemented MS-2 broth. None of the minor discrepancies with gentamicin were corrected by using supplemented medium. However, five of eight very major discrepancies occurring with gentamicin and tobramycin and three of four minor discrepancies occurring with tobramycin and amikacin were corrected, resulting in 92.5% essential agreement for gentamicin and 100% essential agreement for amikacin and tobramycin. In a recent study, (D. A. Schwab, and D. L. Oblack, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C310, p. 363), 6 of 19 gentamicin-resistant *P. aeruginosa* isolates were categorized as susceptible by the MS-2 with unsupplemented MS-2 broth. After cation supplementation of MS-2

broth, all six isolates demonstrated resistance upon retesting.

The results of this study suggest that use of the AMS with GSC-plus cards does not currently provide susceptibility results which agree closely with an established reference procedure, i.e., microdilution MICs. Neither category results nor MICs agreed closely with results of the reference method. However, the convenience and overall user facility of the AMS (1, 6) was confirmed in this study, especially if mating of identification and susceptibility results was employed. In this regard, awaiting EBC-plus identification results does not delay the reporting of mated responses by using the new 4-h identification scheme. However, *P. aeruginosa* may require up to a 10-h incubation period for accurate identification, and thus, reports of susceptibility tests for that organism could frequently be delayed.

The Abbott MS-2 provided more accurate susceptibility results, especially if tests involving *P. aeruginosa* were performed with calcium-supplemented medium. Although the MS-2 does not provide the level of automation achieved by the Vitek, it is not necessary to know the species identity of an isolate for accurate interpretation of susceptibility results. It is necessary only to distinguish between *Enterobacteriaceae* and *P. aeruginosa* to perform susceptibility tests in the MS-2. Furthermore, the routine availability of susceptibility results in 4 to 5 h which has been previously reported (2, 14) was confirmed in the present study.

ACKNOWLEDGMENTS

This study was partially supported by both Abbott Diagnostics Division and Vitek Systems, Inc.

LITERATURE CITED

1. Aldridge, C., P. W. Jones, S. Gibson, J. Lanham, M. Meyer, R. Vannest, and R. Charles. 1977. Automated microbiological detection/identification system. *J. Clin. Microbiol.* 6:406-413.
2. Barnes, W. G., L. R. Green, and R. L. Talley. 1980. Clinical evaluation of automated antibiotic susceptibility testing with the MS-2 system. *J. Clin. Microbiol.* 12:527-532.
3. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility by a standardized single disk method. *Am. J. Clin. Pathol.* 45:493-496.
4. Gavan, T. L., and A. L. Barry. 1980. Microdilution test procedures, p. 459-462. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
5. Gavan, T. L., and D. A. Butler. 1974. An automated microdilution method for antimicrobial susceptibility testing, p. 88-93. In A. Balows (ed.), *Current techniques for antibiotic susceptibility testing*. Charles C Thomas, Publisher, Springfield, Ill.
6. Goldstein, J., J. J. Guarneri, and J. Scherer. 1982. Use of the antimicrobial system for rapid antimicrobial susceptibility testing of *Enterobacteriaceae* in a clinical laboratory. *J. Clin. Lab. Automation* 2:329-337.

7. Hansen, S. L., and P. K. Freedy. 1983. Concurrent comparability of automated systems and commercially prepared microdilution trays for susceptibility testing. *J. Clin. Microbiol.* 17:878-886.
8. Jones, R. N. 1983. Antimicrobial susceptibility testing (AST): a review of changing trends, quality control guidelines, test accuracy, and recommendation for the testing of β -lactam drugs. *Diagn. Microbiol. Infect. Dis.* 1:1-24.
9. Kelly, M. T., J. M. Latimer, and L. C. Balfour. 1982. Comparison of three automated systems for antimicrobial susceptibility testing of gram-negative bacilli. *J. Clin. Microbiol.* 15:902-905.
10. Lampe, M. F., C. L. Aitken, P. G. Dennis, P. S. Forsythe, K. E. Patrick, F. D. Schoenknecht, and J. C. Sherris. 1975. Relationship of early readings of minimal inhibitory concentrations to the results of overnight tests. *Antimicrob. Agents Chemother.* 8:429-433.
11. Murray, P. R., and J. H. Jorgensen. 1981. Quantitative susceptibility test methods in major United States medical centers. *Antimicrob. Agents Chemother.* 20:66-70.
12. National Committee for Clinical Laboratory Standards. 1980. Proposed standard PSM-7. Standard method for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. National Committee for Clinical Laboratory Standards. 1983. Tentative standard M7-T. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. Thornsberry, C., J. P. Anhalt, J. A. Washington II, L. R. McCarthy, F. D. Schoenknecht, J. C. Sherris, and H. J. Spencer. 1980. Clinical laboratory evaluation of the Abbott MS-2 automated antimicrobial susceptibility testing system: report of a collaborative study. *J. Clin. Microbiol.* 12:375-390.
15. Thornsberry, C. and T. L. Gavan. 1980. Automated procedures for antimicrobial susceptibility tests, p. 491-494. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
16. Thornsberry, C., T. L. Gavan, J. C. Sherris, A. Balows, J. M. Matsen, L. D. Sabath, F. Schoenknecht, L. D. Thrupp, and J. A. Washington II. 1975. Laboratory evaluation of a rapid, automated susceptibility testing system: report of a collaborative study. *Antimicrob. Agents Chemother.* 7:466-480.