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Rapid Determination of Minimum Inhibitory Concentrations of Antimicrobial Agents by the Autobac Method: A Collaborative Study

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Four laboratories collaborated in an evaluation of the Autobac minimum inhibitory concentration (MIC) test system. The MICs or ranges of MICs determined in this system were compared with the MICs obtained with a microtube modification of the International Collaborative Study broth dilution technique. A total of 1,260 strains, mostly recent clinical isolates and including multiresistant strains, were tested by the four laboratories against 10 antibiotics; 9,360 separate MIC determinations were made. There was an overall agreement of approximately 95% between the two methods. Levels of agreement below 80% were obtained with only 4 of the 104 antibiotic-species pairs. In only one of the four major organism groups (staphylococci and penicillin G) was agreement less than 85%. There was a symmetrical distribution of MIC differences between the two methods. Tests with 56 selected strains were performed in each of four laboratories in an inter- and intra-laboratory reproducibility study. Both methods showed a standard deviation (both inter- and intra-laboratory) of one-half of a twofold dilution step. The Autobac method was actually less variable than the reference method and had equivalent reproducibility. This was particularly true when the Autobac system was operated so that the results generated permitted calculations of MICs via regression analysis.

The broth dilution test has always been considered a primary laboratory approach to the study of the in vitro antibiotic susceptibilities of particular organisms. It has been thought that broth systems approximate the in vivo situation more closely than disk diffusion systems and that they provide a direct numerical value, the minimum inhibitory concentration (MIC), without any need for conversion or regression lines. They also allow additional studies, such as determinations of the minimum bactericidal concentrations. Disk diffusion tests were introduced mainly because they were less cumbersome technically when large numbers of organisms were tested against many antibiotics.

Mechanization and automation have made broth dilution susceptibility testing much less time consuming, and fluid systems are used almost without exception in advanced, automated tests. At the time of its initial introduction and approval, the Autobac 1 system (Pfizer Diagnostics) was designed to provide data comparable to those obtained in the widely used, standardized agar diffusion test (Kirby-Bauer test) (2, 4), which recognizes three interpretative categories (resistant, intermediate, and susceptible). Because of recent increased interest in determining the MICs of antimicrobial agents, Pfizer Diagnostics has further developed the capability of the Autobac system by modifying the existing equipment to provide automated determinations of the MICs for rapidly growing aerobic and facultatively anaerobic organisms. This report describes an inter-institutional evaluation of this modified system.

MATERIALS AND METHODS

Collaborative study: protocol. The Autobac MIC system and method were evaluated at four laboratories, with coordination provided by the Antimicrobic Investigation Section, Bacteriology Division, Center for Disease Control. The participants were Thomas L. Gavan, Cleveland Clinic Foundation, Fritz D. Schoenknecht, University of Washington, Clyde Thornsberry, Center for Disease Control, and John A. Washington II, Mayo Clinic and Mayo Foundation. The methods and protocols for a two-phase study were agreed upon and followed as closely as possible by the investigators. In phase 1, the accuracy of the Autobac MIC method was assessed relative to a microdilution modification of the International Collaborative Study broth dilution method (3) (called the reference method below). Each of the four laboratories obtained 315 clinical isolates for this test locally. Table 1 shows the species and genus distribution of the isolates utilized by each laboratory. The MICs of ampicillin, carbeni-

cillin, cephalothin, chloramphenicol, gentamicin, kanamycin, penicillin, and tetracycline were determined for all gram-negative isolates excepting *Pseudomonas aeruginosa*, *Acinetobacter* sp., and other *Pseudomonas* sp., against which penicillin G was not tested. MICs of cephalothin, chloramphenicol, clindamycin, gentamicin, methicillin, penicillin G, and tetracycline were measured for the staphylococci. MICs of ampicillin, cephalothin, chloramphenicol, penicillin G, and tetracycline were determined for the enterococci. This resulted in 104 specific antibiotic-species combinations. Each laboratory included resistant organisms among the clinical isolates.

In phase 2 we evaluated both intra- and inter-laboratory reproducibility of the results obtained with the Autobac and reference methods. Four strains from each of the 14 bacterial groups (56 strains in all) (Table 1) were assembled by the coordinating laboratory and distributed to all four participating laboratories in the form of quadruplicate frozen cultures for storage at −70°C. Each of these strains was subjected to parallel MIC determinations by the Autobac and reference methods in each of the four laboratories. The antibiotic panels were the same as those tested in phase 1. Intra-laboratory reproducibility was measured by subjecting each strain to parallel MIC testing with the appropriate antibiotics on 3 different days. In each of the independent tests, the standardized inoculum was derived from a newly frozen culture.

Reagents and media. The lots of media, antimicrobial agents, and disks used in each laboratory were identical. The antibiotic-containing elution disks and their nominal masses are shown in Table 2. They were manufactured by Pfizer Diagnostics, using a new microvolume impregnation-rapid drying technique. A stock solution of each antimicrobial agent was prepared volumetrically from bulk assay powder and diluted 1:2, 1:4, 1:8, and 1:16. With a precision delivery system, prepunched, absorbent paper disks (diameter, 6 mm) were individually impregnated with 10 μl of each concentration of antimicrobial agent. The disks were immediately subjected to rapid drying by passage through a gently heated wind tunnel; they were then placed in vials that were hermetically sealed and stored in a freezer at −20°C before use.

TABLE 1. *Genus and species distribution of the 315 bacterial isolates utilized by each laboratory in phase 1 of the Autobac MIC system evaluation*[a]

Organism	No. of isolates
S. aureus	40
S. epidermidis	20
Enterococci	20
E. coli	40
Klebsiella sp.	30
Enterobacter sp.	20
Proteus mirabilis	20
Other *Proteus* sp.	15
Serratia sp.	20
Citrobacter sp.	15
Providencia sp.	15
P. aeruginosa	30
Other *Pseudomonas* sp.	15
Acinetobacter calcoaceticus	15

[a] Four isolates from each category were selected for testing in all laboratories in phase 2.

TABLE 2. *Antibiotic disk masses of the Autobac MIC test when breakpoint analysis was used*

Antibiotic	Nominal disk masses[a]	Final concn (μg/ml)
Ampicillin	3[b], 12, 48[b]	2, 8, 32
Carbenicillin	12[b], 48, 192[b]	8, 32, 128
Cephalothin	3[b], 12, 48[b]	2, 8, 32
Chloramphenicol	12[b], 24, 48[b]	8, 16, 32
Clindamycin	1.5[b], 3, 6[b]	1, 2, 4
Gentamicin	3[b], 6, 12[b]	2, 4, 8
Kanamycin	6[b], 12, 24[b]	4, 8, 16
Methicillin	3[b], 6, 12[b]	2, 4, 8
Penicillin G (staphylococci)	0.188[b], 3, 48[b]	0.13, 2, 32
Penicillin G (other organisms)	3, 12[b], 48[b]	2, 8, 32
Tetracycline	3[b], 6, 12[b]	2, 4, 8

[a] Each disk mass was eluted into 1.5 ml of broth containing standardized inoculum. Units for all antibiotics except penicillin, micrograms; units for penicillin, international units.

[b] The two disk masses used for regression analysis for each antibiotic.

By using ^{131}I, this impregnation system was shown to have a mean precision of ±3% (±2 standard deviations; range, 2.4 to 3.6%). Overall variation for 95% of the disks was ±5% (±2 standard deviations). Each of 31 lots of elution disks was also subjected to a bioassay. The contents showed a variability from ±3.4 to ±14.6% and, in general, were slightly high. Three of the antibiotic powders were manufactured by Pfizer; the remaining seven were obtained from other manufacturers.

The media used were Mueller-Hinton broth (lot 627274; Difco Laboratories) supplemented with reagent-grade $CaCl_2$ (anhydrous) and reagent-grade $MgCl_2 \cdot 6H_2O$ (7) to give final concentrations of 0.06 g of calcium per liter and 0.03 g of magnesium per liter, Tryptic soy broth (lot 75902; Pfizer Diagnostics), and Autobac MIC broth (lot 72667 B; Pfizer Diagnostics). The MIC broth was not the eugonic broth used in the present interpretative Autobac method but rather sterile, optically clear broth containing the following (in grams per liter): acid hydrolysate of casein, 17.5; brain heart infusion, 4.0; dextrose, 2.0; total calcium, 0.06 (by addition of reagent-grade $CaCl_2$, anhydrous); and total magnesium, 0.03 (by addition of reagent-grade $MgCl_2 \cdot 6H_2O$). The pH was 7.3 ± 0.2. The standardized initial inocula were prepared in Autobac 1 inoculum standardization solution (Pfizer Diagnostics), which contained 4.2 g of sodium chloride per liter, 3.2 g of dibasic potassium phosphate per liter, and 1.6 g of monobasic potassium phosphate per liter; the pH was 7.0 ± 0.2.

Organisms. The majority of the 315 organisms selected by each investigator for phase 1 studies (Table 1) were recent clinical isolates; these were supplemented with specific stock cultures and included multiresistant strains. Thus, quotas of each of the species varying with respect to MICs were achieved. The percentages of stock cultures used in the four laboratories varied from 5.5 to 13%. Stock cultures were

generally stored frozen in blood or serum and were not subcultured during storage. The recent clinical isolates had undergone one to five subcultures on Trypticase soy blood agar (average, two subcultures) before examination in this study. In addition to these organisms, the following control strains were also employed by the investigators throughout both phases of the study: *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

Susceptibility test methods. (i) Reference MIC method. A microdilution modification of the broth dilution method recommended by Ericsson and Sherris (3) was used as the reference method in this study. Appropriate antibiotic dilutions prepared in cation-supplemented Mueller-Hinton broth were automatically dispensed with an MIC-2000 inoculator (Dynatech Laboratories) into the wells of a plastic microtray. These trays were stored at −20°C for no longer than 1 month in sealed containers until use, at which time they were thawed and each well was inoculated by using the MIC-2000 inoculator. The final inoculum concentration was 10^5 colony-forming units per ml (or 10^4 colony-forming units per well). The trays were then covered and incubated for 16 to 20 h at 36°C. Endpoints were read with the unaided eye by using front lighting against a black background.

(ii) Autobac MIC method. The Autobac MIC test system consisted of instrumentation, a disposable plastic cuvette, and several reagents. Some of these components were identical or similar to the components of the original Autobac method. Only two of the three disk masses were employed in the regression analysis version (see below) of this method. The elution disk masses were separated by two- or fourfold dilution steps, as required to encompass concentrations of therapeutic interest. The elution disks listed in Table 2 were dispensed at the initiation of the test, after which a tube of MIC broth containing the photometrically standardized inoculum was screwed into the test tube port of the cuvette. By means of channels built into the cuvette, the broth inoculum was then distributed equally into the control chamber and all 12 test chambers of the cuvette. The antibiotic from each disk was eluted rapidly into the 1.5 ml of broth inoculum contained in each test chamber. At the end of a 5-h incubation period at 36°C in an Autobac incubator-shaker, the cuvette was transferred manually to an Autobac Multi-Test System (MTS) photometer, and the growth in each test chamber of the cuvette was evaluated optically. Antibiotic effectiveness was measured as the growth differential between organisms in chambers receiving antibiotics and those in the control chamber.

The Autobac MTS photometer is similar to the original Autobac photometer except for a selector panel; the installation of this panel allows the Autobac MTS photometer to be used for the original interpretative susceptibility testing, for MIC testing, and for other tests planned for this system. The Autobac photometer performed the following functions. (i) It was used in the standardization of the starting inoculum. The MTS photometer could be programmed to handle an inoculum of either 10^7 or 10^8 colony-forming units per ml of inoculum standardization solution, as required in the MIC testing protocol. (ii) It was also used in the evaluation of the antibiotic effect. This was done automatically by the photometer. A light scattering index (LSI), which ranged from 0 for no inhibition of growth to 1.0 for complete growth inhibition, was used to calculate the result of either interpretative or MIC testing, according to the test method selected. The LSI is defined as follows: $LSI = (G_c - G_x)/(G_c - G_k)$, where G_c is the logarithm of the 35° angle light scattering intensity for the uninhibited control after incubation, G_k is the logarithm of the 35° angle light scattering intensity for the uninhibited control before incubation, and G_x is the logarithm of the 35° angle light scattering intensity for the antimicrobial-challenged sample after incubation. Both the original Autobac 1 photometer and the new Autobac MTS photometer operate on the same principle. In the Autobac interpretative susceptibility test, LSIs were automatically calculated, compared with a breakpoint LSI of 0.60, and then printed out along with an interpretation of either resistant, intermediate, or susceptible. In MIC testing, the photometer automatically calculates and prints out the LSIs, which are then manually coverted to MICs by using tables or a convenient slide rule set. Alternatively, the Autobac MIC computer option, consisting of a keyboard with digital display and an alpha-numeric auxilliary printer, can be used in conjunction with the photometer. This expanded system allows a user to enter various codes, which represent a variety of possible antimicrobial panels being tested, along with an accession number and isolate number. For each cuvette containing a test organism that is read, a microprocessor in the MTS photometer automatically computes and prints out the MICs of the antibiotics included in the antimicrobial panel.

MICs resulting from the light scattering data of the Autobac method were obtained in this study by two different procedures, breakpoint analysis and regression analysis. The breakpoint analysis method used the more traditional light scattering endpoints, or breakpoints, to determine the MICs by comparing the light scattering measured in the presence of each of the three concentrations of each antibiotic used with the threshold or breakpoint LSIs that had been established experimentally in previous preclinical trials. For instance, the LSI breakpoint value used to determine inhibition of staphylococcal growth in the presence of tetracycline was 0.70 for all three concentrations of the antibiotic. In contrast, the regression analysis method, which is discussed elsewhere (5), used empirically derived regression equations to compute the MICs from the LSIs obtained with two of the three antibiotic concentrations. Autobac MIC values obtained by using these two analysis procedures were compared with the reference method MIC values collected in parallel. This enabled an examination of the accuracy (phase 1) and reproducibility (phase 2) of the Autobac MICs derived from both methods of analysis relative to the reference MIC values.

In addition to using multiple concentrations of antibiotics, the Autobac MIC test method differed from the original Autobac interpretative test procedure in the following ways. (i) Two starting broth inoculum concentrations were used, 10^6 colony-forming units per ml when gram-negative organisms were tested with

β-lactam antibiotics and 10^7 colony-forming units per ml for all other antibiotic-organism combinations. (ii) The results for each antibiotic were interpreted differently according to the following four organism classes: staphylococci, enterococci, *Enterobacteriaceae*, and *Pseudomonas* and *Acinetobacter* species.

Data transmission and tabulation. A silent model 733-ASR data terminal (Texas Instruments) with a tape deck was interfaced with the Autobac photometer and was used to collect, store, and transmit all Autobac data. Parallel data collected with the reference method were entered manually on the terminal as the Autobac data were sent by telephone to the Pfizer PDP-10 computer (Digital Equipment Corp.) in Groton, Conn. The previously taped Autobac data were sent batched. Multiple transmissions were performed to enable identity comparisons, ruling out random line noise errors. Finally, original data sheets of all results transmitted by telephone were mailed to Pfizer Diagnostics so that they could be compared with earlier telephone transmissions.

RESULTS

In phase 1, the four investigators performed a total of 9,360 MIC determinations on 1,260 bacterial strains with the Autobac MIC method and the same number with the reference method. These determinations involved 104 antibiotic-species pairs, with each investigator testing the same number of strains within each species (14 strains in all) and the same panels of antibiotics. As Table 3 shows, overall comparisons of the 9,360 MIC determinations conducted by the two methods showed that there was approximately 95% agreement between the Autobac MICs and the reference MICs, allowing for a ±1 twofold dilution error of the reference broth dilution method. Agreement with the reference method was slightly higher when regression analysis was used to derive the Autobac MIC than when breakpoint analysis was used (95.3 versus 94.7%).

Agreement between the reference and Autobac MIC methods for the four major groups of organisms (i.e. staphylococci, enterococci, *Enterobacteriaceae*, and the *Pseudomonas* sp.-*Acinetobacter* sp. group) was in the 94 to 96% range (Table 3). Of the 27 antibiotic-major organism pairs tested by all investigators, 26 (96.3%) had agreement levels of 85% or greater when the Autobac regression analysis method was compared with the reference method. In only one case was the agreement less than 85% (penicillin G and staphylococci [84%]). Of the 26 antibiotic-organism pairs tested with ≥85% agreement, 24 (92.3%) had agreement levels of 90% or more, and of these 24, 18 (75%) had agreement levels of ≥95%. A similar comparison of the Autobac breakpoint method with the reference method yielded slightly lower levels of agreement.

Table 4 shows that the Autobac breakpoint and regression methods also exhibited very high agreement with the reference method when the 14 individual species were considered with regard to all antibiotics. Table 5 shows that for 99 of the 104 individual antibiotic-species combinations tested (95.2%), there were agreement levels of at least 85% between the results of the Autobac regression method and those of the reference method. Of these 99 pairs, 91 (91.9%) had agreement levels of at least 90%, and 74 (91.3%) had agreement levels of at least 95%. A similar comparison of the Autobac breakpoint and reference methods showed slightly lower

TABLE 3. *Percent agreement within ± 1 twofold dilution between the MIC values obtained with the Autobac MIC method and the reference method for each antibiotic (phase 1)*

Antibiotic	% Agreement within ±1 twofold dilution							
	Staphylococci		Enterococci		*Enterobacteriaceae*		*Pseudomonas* and *Acinetobacter* species	
	Regression[a]	Breakpoint[b]	Regression	Breakpoint	Regression	Breakpoint	Regression	Breakpoint
Ampicillin			99	98	90	92	95	95
Carbenicillin					96	95	86	88
Cephalothin	98	98	88	86	94	94	99	100
Chloramphenicol	100	98	98	98	98	98	97	95
Clindamycin	97	98						
Gentamicin	94	94			99	98.4	95	98
Kanamycin					97	93	98	98
Methicillin	92	94						
Penicillin G	84	80	92	92	97	97		
Tetracycline	95	95	99	98	94	91	97	96
Avg[c]	94	94	95	94	96	95	95	95

[a] Regression analysis was used to convert LSI values to MIC values in the Autobac method.
[b] Breakpoint analysis was used to convert LSI values to MIC values in the Autobac method.
[c] Averages for all antibiotics and organisms: regression analysis, 95.3%; breakpoint analysis, 94.7%.

levels of agreement (i.e., 92.3% of the 104 antibiotic-species pairs had agreement levels of ≥85%). Only four antibiotic-species pairs had levels of agreement below 80% when the Autobac regression method was compared with the reference method, and in these cases, usually one laboratory was the source of the discrepancy.

TABLE 4. *Percent agreement within ± 1 twofold dilution between the MIC values obtained with the Autobac and reference MIC methods by all four investigators for each species (all antibiotics)*

Species	No. of tests	% Agreement within ±1 twofold dilution	
		Regression[a]	Breakpoint[b]
S. aureus	1120	95.8	94.5
S. epidermidis	560	90.7	93.0
Enterococci	400	95.0	94.3
E. coli	1280	97.8	96.6
Klebsiella sp.	960	96.8	96.8
Enterobacter sp.	640	91.3	92.5
Proteus mirabilis	640	95.2	93.0
Other *Proteus* sp.	480	95.8	91.9
Serratia sp.	640	97.0	95.5
Citrobacter sp.	480	95.6	93.3
Providencia sp.	480	94.4	95.2
P. aeruginosa	840	97.5	96.8
Other *Pseudomonas* sp.	420	91.2	92.6
Acinetobacter sp.	420	95.7	94.5
Total	9,360	95.3	94.7

[a] Regression analysis was used to convert LSI values to MIC values in the Autobac method.
[b] Breakpoint analysis was used to convert LSI values to MIC values in the Autobac method.

TABLE 5. *Number of antibiotic-species pairs by percent agreement within ±1 twofold dilution ranges: Autobac MIC versus reference MIC*[a]

% Agreement	No. of pairs within ± 1 twofold dilution[a]	
	Autobac breakpoint analysis	Autobac regression analysis
>95	72	74
90–94.9	15	17
85–89.9	9	8
80–84.9	3	1
<80	5	4

[a] A total of 104 antibiotic-species pairs were tested.

The distribution of the differences in the MICs obtained with the Autobac and reference methods is an important aspect of the phase 1 results. The results obtained with cephalothin against the four major organism catagories are shown in Table 6 as an example. The distribution is symmetrical, without obvious trends in either direction. Table 7 shows the actual distribution of the MIC values obtained by the Autobac regression analysis and reference methods for cephalothin. Similar comparisons for all other antibiotics revealed no major differences.

The upper and lower limits of the MIC values given by any dilution method are largely a function of the minimum and maximum concentrations of each antimicrobial agent employed. Beyond these limits, the MIC is expressed as less than or equal to the lowest antimicrobial concentration and greater than the highest concentration. Approximately 80% of the organisms tested in this study had MICs that were in these off-scale regions by either the Autobac or reference method or both. In the remaining 20%, MIC values measured by both the Autobac and reference methods were on scale. In calculating the percent agreement between methods for this population, it must be recognized that Autobac elution disk masses for penicillin, carbenicillin, cephalothin, and ampicillin were separated by fourfold and not twofold dilution steps (e.g., for cephalothin 2, 8, and 32 μg/ml). Consequently, agreement with the reference method within one twofold dilution step for these four antibiotics is defined as agreement within one dilution step of the MIC ranges reported by the Autobac method (e.g., MIC values of 2, 4, 8, and 16 μg/ml by the reference method are all considered within one twofold dilution step of the 4- to 8-μg/ml range reported by the Autobac method for cephalothin). The results of these calculations are shown in Table 8.

Only small decreases in overall percent agree-

TABLE 6. *Distribution of differences between the MIC values obtained with the Autobac (regression analysis) and reference methods by all four investigators for cephalothin*

Organism	No. of strains	% Incidence of twofold MIC differences between Autobac and reference MIC methods[a]										
		≥−5	−4	−3	−2	−1	0	+1	+2	+3	+4	≥+5
Staphylococci	240	0.4	0.0	0.4	0.0	0.4	95.8	1.3	1.3	0.0	0.0	0.4
Enterococci	80	0.0	0.0	0.0	8.8	8.7	71.3	7.5	2.5	1.2	0.0	0.0
Enterobacteriaeae	700	0.1	0.0	0.6	3.4	10.0	76.9	7.1	1.1	0.6	0.1	0.0
Pseudomonas sp. and *Acinetobacter* sp.	240	0.0	0.0	0.0	0.8	0.8	97.1	1.3	0.0	0.0	0.0	0.0
Total	1,260	0.2	0.0	0.4	2.6	6.3	84.0	4.9	1.0	0.4	0.1	0.1

[a] A minus sign means that the Autobac MIC was less than the reference MIC (e.g., 0.6 in the −3 column means that 0.6% of the organisms tested had Autobac MIC values 3 twofold dilutions lower than the reference MIC values).

TABLE 7. *Distribution of cephalothin MIC values as obtained by all four investigators for the major organism categories tested*

Reference MIC (μg/ml)	No. of strains with Autobac regression analysis MICs of:											
	≤2 μg/ml		4 to 8 μg/ml		16 to 32 μg/ml			>32 μg/ml				
	Staphylococci	*Enterobacteriaceae*	Staphylococci	*Enterobacteriaceae*	Staphylococci	*Enterobacteriaceae*	Enterococci	*Pseudomonas* and *Acinetobacter* spp.	Staphylococci	*Enterobacteriaceae*	Enterococci	*Pseudomonas* and *Acinetobacter* spp.
≤1	216	11		2					1			
2	8	72	3	27		3						
4	1	34	1	116	3	3				1		
8		5	1	67		11				1	1	
16				16		14	15	1		3	2	
32				12		19	34			12	6	3
64	1	1	1	4		20	7	2	2	25	4	5
>64						7	7	2	2	214	4	227

ment, as well as in the results for individual antibiotics, occurred relative to the analogous percentages of agreement when all strains (i.e., on scale and off scale) were used. The Autobac (regression analysis) comparison with the reference method averaged 93% for this on-scale subset of tests, ranging from 90% (kanamycin) to 96.5% (chloramphenicol). Similarly, the Autobac breakpoint analysis yielded approximately the same accuracy of agreement with the reference values for this on-scale subset of strains.

In Table 9, the percent agreement from laboratory to laboratory is shown for each antibiotic and each investigator. The methods compared are the Autobac MIC regression analysis method and the reference method. The variation from laboratory to laboratory was negligible.

Statistical analyses of the phase 2 data (Tables 10 and 11) indicated that ±1 standard deviation (both intra- and inter-laboratory) of all three methods was generally less than one-half of a twofold dilution (Table 10). In terms of intra-laboratory considerations, the Autobac breakpoint analysis method had variability and reproducibility equivalent to the reference method, whereas in terms of inter-laboratory consideration it had equivalent reproducibility and significantly less variability compared with the reference method. The analogous comparisons of the Autobac regression analysis method with the reference method showed that from both intra- and inter-laboratory aspects, the Autobac method was significantly less variable and of equivalent reproducibility to the reference method. This version of the Autobac method was judged to be less variable and more reproducible than the breakpoint version.

A comparison of results obtained with the Autobac regression analysis and reference MIC methods is shown in Table 12. In this comparison, resistance was defined as follows: ampicillin MIC, >16 μg/ml; carbenicillin MIC, ≥192 μg/ml; cephalothin MIC, >16 μg/ml; chloramphenicol MIC, >16 μg/ml; clindamycin MIC, >4 μg/ml; gentamicin MIC, >5 μg/ml: kanamycin MIC, >16 μg/ml; methicillin MIC, >6 μg/ml; penicillin G MIC with staphylococci, >0.125 U/ml; penicillin G MIC with gram-negative organisms, >16 U/ml; and tetracycline MIC, >8 μg/ml. These definitions were based on determinations by the reference method. Multidrug resistance was arbitrarily defined as follows: staphylococci, resistant to three or more of the seven antimicrobial agents in the panel; *Enterobacteriaceae*, resistant to five or more of the eight antimicrobials in the panel; and nonfermentative gram-negative bacteria, resistant to six or more of the seven antimicrobials in the panel. Comparable results

TABLE 8. *Percentage of on-scale strains with MIC values in agreement within ± 1 twofold dilution, as tested by all four investigators: Autobac regression analysis MIC versus reference MIC*[a]

Antibiotic	No. of strains tested	No. of strains on scale by both MIC methods[a]	No. of strains within ±1 twofold dilution for on-scale strains[a]
Ampicillin	1,020	324 (31.8)[b]	294 (90.7)[b]
Carbenicillin	940	276 (29.4)	256 (92.8)
Cephalothin	1,260	380 (30.2)	354 (93.2)
Chloramphenicol	1,260	173 (13.7)	167 (96.5)
Clindamycin	240	0 (0)	NA[c]
Gentamicin	1,180	140 (8.8)	99 (95.2)
Kanamycin	940	20 (2.1)	18 (90.0)
Methicillin	240	18 (7.5)	17 (94.4)
Penicillin G[d]	1,020	389 (38.1)	364 (93.6)
Tetracycline	1,260	148 (11.7)	136 (91.9)

[a] The MIC obtained by both the Autobac and reference methods was on scale.
[b] Numbers in parentheses are percentages.
[c] NA, Not applicable.
[d] For staphylococci tested with penicillin G, ±1 fourfold dilution was used.

TABLE 9. *Percent laboratory to laboratory agreement (within ± 1 twofold dilution) between Autobac 1 MIC values (using regression analysis) and the reference method: phase 1*

Antibiotic	Gavan	Schoenknecht	Thornsberry	Washington	Avg
Ampicillin (n = 255)	93.7	95.3	90.6	89.0	92.2 (n = 1,020)
Carbenicillin (n = 235)	93.2	94.9	91.5	94.5	93.5 (n = 940)
Cephalothin (n = 315)	94.6	95.9	96.5	94.0	95.2 (n = 1,260)
Chloramphenicol (n = 315)	98.1	97.1	98.7	98.4	98.1 (n = 1,260)
Clindamycin (n = 60)	100	98.3	90.0	100	97.1 (n = 240)
Gentamicin (n = 295)	99.3	97.6	96.3	94.6	96.9 (n = 1,180)
Kanamycin (n = 235)	97.0	97.4	95.7	99.1	97.3 (n = 940)
Methicillin (n = 60)	93.3	96.7	93.3	85.0	92.1 (n = 240)
Penicillin G (n = 255)	94.1	91.8	94.9	94.1	93.7 (n = 1,020)
Tetracycline (n = 315)	93.3	91.1	96.8	98.4	94.9 (n = 1,260)
Avg (n = 2,340)	95.5	95.2	95.2	95.2	95.3 (n = 9,360)

were obtained in all four laboratories, with the total percentage of multidrug-resistant strains ranging from 16.8 to 23.8%. The data shown in Table 12 involve almost 21% of the phase 1 strains. The levels of agreement with these subsets of organisms are nearly identical to those obtained with all phase 1 strains. The low agreement with staphylococci and penicillin represents discrepancies with 13 strains, 12 of which gave resistant MIC values by both methods. In other words, the discrepancies were due to different dilution schedules and endpoints and did not result in interpretive errors.

DISCUSSION

The system under investigation was basically the same as the Autobac 1 system, which was subjected to an earlier collaborative study (8). Although this equipment can be used in the original interpretative susceptibility testing mode, it can also generate MIC information for 10 selected antibiotics within a 5-h incubation period. The necessary equipment modifications to existing versions of the Autobac 1 can be done in the field. It is this MIC reporting capability that was under investigation during our study.

The Autobac MIC results for the 10 antibi-

TABLE 10. *Estimates of standard deviations of MICs (log_2 scale): phase 2*[a]

Comparison	Standard deviation	
	All antibiotics (n = 4,992)	Range
Intra-laboratory		
Reference method	0.399 ± 0.010[b]	0.309 ± 0.021–0.536 ± 0.040
Autobac breakpoint method	0.391 ± 0.010	0 ± 0–0.537 ± 0.040
Autobac regression analysis method	0.354 ± 0.009	0.205 ± 0.036–0.465 ± 0.034
Inter-laboratory		
Reference method	0.552 ± 0.013	0.404 ± 0.026–0.804 ± 0.134
Autobac breakpoint method	0.474 ± 0.011	0 ± 0–0.670 ± 0.049
Autobac regression analysis method	0.476 ± 0.011	0.360 ± 0.060–0.587 ± 0.040

[a] Estimates were derived from a linear model fitted separately for every possible antibiotic-species combination (pooling over strains).
[b] Mean ± standard error.

TABLE 11. *Percentages of MICs in agreement with the most frequently observed value for each method, antibiotic, species, and strain (phase 2)*

Comparison	% In agreement	
	All antibiotics	Range
Intra-laboratory		
Reference method	89.9	85.1–98.9
Autobac regression analysis method (reference scale)[a]	89.7	85.4–94.4
Reference method (Autobac breakpoint scale)[b]	95.3	88.5–97.9
Autobac breakpoint method	94.8	91.8–100.0
Autobac regression analysis method (Autobac breakpoint scale)[b]	95.3	93.1–97.7
Inter-laboratory		
Reference method	86.6	75.0–100.0
Autobac regression analysis method (reference scale)[a]	86.2	80.7–92.0
Reference method (Autobac breakpoint scale)[b]	93.5	81.2–100.0
Autobac breakpoint method	92.9	90.1–100.0
Autobac regression analysis method (Autobac breakpoint scale)[b]	93.3	92.1–100.0

[a] Scale converted to reference MIC scale.
[b] Scale converted to Autobac breakpoint MIC scale.

otics had 92 to 98% agreement (average, 95%) within ±1 twofold dilution with a microtube version of the International Collaborative Study broth dilution method. These levels of agreement were common to all four laboratories. Because of the different result ranges with the Autobac and reference methods, the MIC values for the two methods in the off-scale regions could not be compared directly. The data on the approximately 20% of the organisms with MICs on scale by both methods were analyzed separately and found to have an average level of 93.1% agreement. None of the individual antibiotics had less than 90% agreement within this subset of data. This strengthens the validity of the percentage agreement obtained for the full set of test organisms with either on-scale or off-scale MICs. As new approaches are being used for in vitro susceptibility testing, differences in methodology are unavoidable, and direct comparisons between two methods may not always be possible on a point for point basis. It is particularly important to analyze portions of the results where direct comparisons are possible.

Since both methods examined in this study were based on broth dilution techniques, the attempt to correlate results was substantially easier than in the original collaborative study

TABLE 12. *Percent agreement (within ± 1 twofold dilution) for multiresistant bacteria (all four laboratories): Autobac regression versus reference MICs*

Antibiotic	% Agreement		
	Staphylococci (n = 32)	*Enterobacteriaceae* (n = 128)	Nonfermentative gram-negative bacteria (n = 103)
Ampicillin		98.4	97.1
Carbenicillin		96.9	85.4
Cephalothin	84.3	95.3	100
Chloramphenicol	96.9	96.9	99.0
Clindamycin	96.9		
Gentamicin	87.5	96.9	95.1
Kanamycin		95.3	100
Methicillin	81.3		
Penicillin G	59.4	100	
Tetracycline	90.6	96.1	99.0
Avg	85.3	97.0	96.5

[a] The average percent agreement for all strains and all antibiotics was 95.5%.

(8), in which disk diffusion, agar dilution, and Autobac 1 (broth based) procedures were studied and compared. Nevertheless, even different broth dilution methods lead to variations in results that may be related to specific methodology (9). Thus, Barry et al. (1) reported that microdilution MICs for gram-negative bacilli are usually 1 $\log_2$ dilution step lower than macrodilution MICs. Analysis of all of the phase 1 data from this study did not reveal a trend in either direction when the rapid Autobac MIC values were compared with the MIC values of the reference method.

All of the problems pertinent to applications of the Autobac MIC method have not been resolved by the current study. First, only 10 antibiotics were evaluated, although additional clinical studies with amikacin, tobramycin, cefoxitin, and cefamandole are presently in progress. Second, there is no explanation for the disagreements encountered with certain antibiotic-organism combinations, especially with penicillin G and staphylococci (particularly *Staphylococcus epidermidis*). These discrepancies may be due to the number of staphylococci which produce low levels of β-lactamase. If so, a possible solution would be the determination of the presence of β-lactamase by a rapid spot or capillary test in those staphylococci with penicillin MICs of ≤0.2 μg/ml. Third, the need for two cuvettes for the two inocula employed in testing gram-negative bacteria with both β-lactam and non-β-lactam antibiotics will add to the cost of susceptibility testing, which may be a problem for some laboratories.

With regard to media, it has become obvious that agar-based susceptibility testing of *P. aeruginosa* against aminoglycosides is subject to variations related to the contents of cations and other components in the agar base (6). Because of the absence of agar, broth dilution techniques, such as the Autobac MIC method, are less subject to these variations. Furthermore, the speed (5-h incubation) at which results of high reliability are obtained is another point in favor of the Autobac MIC method.

The regression analysis approach (5) of the Autobac MIC method is of particular interest. From the data presented in this study (Tables 10 and 11), it is apparent that the range (among antibiotics) of variability tended to be smaller when results based on regression analysis were compared with the reference method. In view of the combined results in phases 1 and 2 of this study, we conclude that the rapid Autobac method, when regression analysis is used, is accurate relative to the reference method for each antibiotic tested and that the Autobac method has significantly less overall variability than the reference method and comparable reproducibility for the 10 antibiotics studied.

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