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Simple Inoculum Standardizing System for Antimicrobial Disk Susceptibility Tests

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A collaborative study was undertaken to evaluate a simple, convenient device which expedites inoculum standardization for antimicrobial disk susceptibility tests. The Inocupac system (Minnesota Mining & Manufacturing [3M] Co., St. Paul, Minn.) was used to perform disk tests in parallel with the standard Bauer-Kirby method. Five investigators tested 100 selected isolates, each in triplicate. Inter- and intralaboratory precision of both disk procedures was essentially comparable. The Inocupac system failed to consistently provide satisfactory growth with some streptococci, but when testing common gram-negative bacilli, staphylococci, and most enterococci, the Inocupac system gave zones about the same size (±2 mm) as the Bauer-Kirby procedure. Interpretive agreement between the two test systems varied from 90 to 99% with different antimicrobial agents, and repeated tests with the Bauer-Kirby method demonstrated the same degree of interpretive agreement. The Inocupac system is a valid alternative method for inoculating disk susceptibility tests.

The single disk, agar diffusion method for determining bacterial susceptibility to antimicrobial agents has been carefully standardized (1, 7, 9). The most widely recognized testing procedure is the method of Bauer et al. (6), often referred to as the Bauer-Kirby or Kirby-Bauer method. The agar overlay method of Barry et al. (3) has been recognized as an acceptable alternative method for testing common, rapidly growing bacterial pathogens (7, 9). With either method, the procedure for standardizing inoculum density is one of the most critical steps to be controlled (1). The present report describes another alternative method for adjusting inoculum density.

With the Bauer-Kirby method, an actively growing broth culture is diluted until the turbidity matches that of a MacFarland 0.5 $BaSO_4$ standard (ca. 10^8 colony-forming units [CFU]/ml). Because this step is rather subjective and time consuming, Barry et al. (3) developed an alternative method which does not require adjustment of turbidity. With the alternative agar overlay method, the inoculum is adjusted by allowing broth cultures to reach the stationary phase of growth, at which time the number of viable cells will approach 10^9 CFU/ml. Most rapidly growing pathogens will approach the stationary phase after 4 to 6 h when 0.5 ml of brain heart infusion broth is inoculated with four to five isolated colonies (1, 3). A simple 1:10 dilution of such small-volume broth cultures should provide an inoculum which is nearly the same as that obtained with a $BaSO_4$ turbidity standard. The agar overlay method of Barry et al. (3) has been found to be acceptable for testing enteric bacilli, *Pseudomonas* sp., *Staphylococcus aureus*, and many enterococci (2) but not for testing other streptococci or genera with less predictable growth rates.

The Inocupac system represents another alternative for adjusting inoculum density without visual adjustment of turbidity. The system was recently developed by investigators at the Minnesota Mining & Manufacturing (3M) Co. (St. Paul, Minn.). It contains a simple broth medium of limited nutritive capacity. The bacterial inoculum quickly reaches the stationary phase of growth and maintains a cell density of about 10^8 CFU/ml (R. L. Nelson, M. W. Downing, J. H. Wicks, M. K. Samoszuk, and B. Hapke, Abstr., Annu. Meet. Am. Soc. Microbiol. 1978, C114, p. 296). Amsterdam (Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C115, p. 296) found that this device yields fairly good interpretive agreement with the standard Bauer-Kirby method.

The present report summarizes the result of a collaborative study which compares the results

of disk tests with the Inocupac system to those obtained with the Bauer-Kirby method. Tests were performed with 100 selected isolates, demonstrating the wide variety of growth characteristics likely to be encountered in clinical laboratory work. Included were some strains likely to challenge the accuracy and precision of both disk methods.

MATERIALS AND METHODS

Bacterial strains. Study strains were selected and distributed by C. Thornsberry and C. N. Baker (Center for Disease Control, Atlanta, Ga.). The 100 isolates included 25 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 12 *Proteus mirabilis*, 1 *Proteus vulgaris*, 2 *Proteus rettgeri*, 2 *Proteus morganii*, 3 *Providencia stuartii*, 1 *Providencia alcalifaciens*, 3 *Serratia marcescens*, 1 *Serratia rubidaea*, 1 *Enterobacter cloacae*, 1 *Enterobacter hafniae*, 1 *Citrobacter diversus*, 1 *Salmonella enteritidis*, 1 *Acinetobacter calcoaceticus* subsp. *anitratus*, 5 *Pseudomonas aeruginosa*, 8 *Staphylococcus aureus*, 2 *Staphylococcus epidermidis*, 4 *Streptococcus faecalis*, 3 *Streptococcus faecium*, 1 *Streptococcus durans*, 1 *Streptococcus bovis*, 1 *Streptococcus mutans*, 1 *Streptococcus pneumoniae*, and 4 beta-hemolytic streptococci (one each of groups A, B, C, and G). In addition, *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) were distributed for quality-control purposes.

Bauer-Kirby disk tests. Five investigators tested each microorganism on 3 separate days by the standardized disk technique of Bauer et al. (6), as defined by the National Committee for Clinical Laboratory Standards (7). Mueller-Hinton agar plates and antimicrobial disks were all provided from a single source. After overnight incubation, the test plates were held against a dark background and were illuminated with reflected light. The diameters of the zones of inhibition were measured to the nearest whole millimeter by holding a ruler or calipers against the back of the petri plate. When testing the streptococci, defibrinated sheep blood (5% vol/vol) was added to the agar medium, and consequently, zone measurements were made from the surface. Each investigator was instructed to measure the zone with no obviously visible growth, ignoring a faint haze or barely visible colonies just inside of an otherwise well-defined zone of inhibition. Also, with swarming *Proteus* spp., a thin veil of swarming growth inside of an otherwise definite zone of inhibition was to be ignored.

Inocupac system. Each time a standard Bauer-Kirby test was inoculated, a second disk test was performed, using an inoculum developed in an Inocupac unit. The Inocupac system consists of a capped vial containing an inoculator and one of two broth media in a crushable glass vial (Fig. 1). The medium designated for use with gram-negative bacilli is described by the manufacturer as containing 0.08% peptone, 0.003% carbohydrates, 0.5% sodium salts, and 0.2

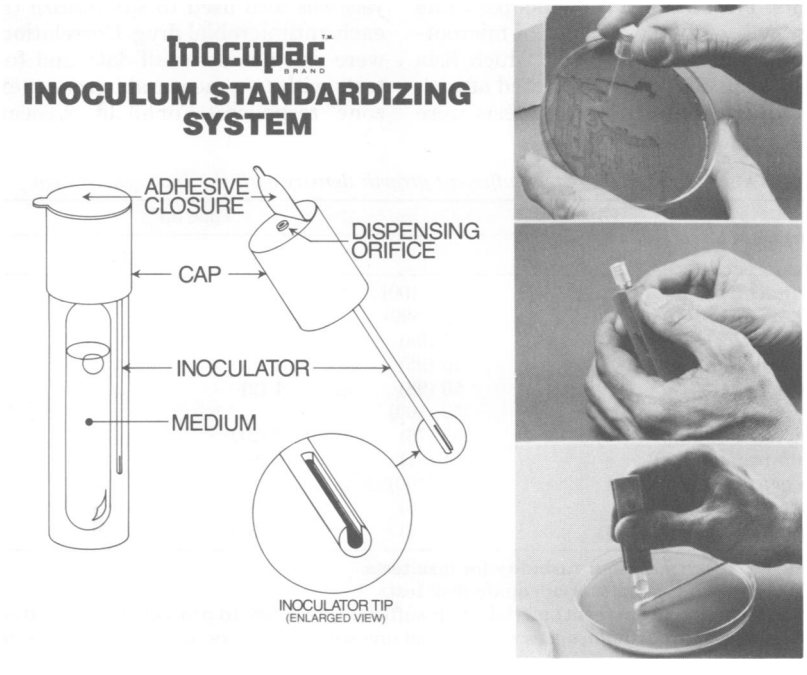

FIG. 1. *Utilization of the Inocupac system. The inoculator is used to select growth from four to five colonies (upper right). The inoculator is replaced, and the ampoule is then crushed (center right) to release the medium. After 4 to 6 h at 35°C, a swab is saturated with 4 to 6 drops of the culture, dispensed through the dispensing orifice (lower right). The plate is then inoculated and disks are applied as with the standard Bauer-Kirby method.*

M phosphate buffer (pH 7.1). The medium designated for testing gram-positive cocci is described as containing 0.008% soytone, 0.02% peptone, 0.01% yeast extract, 0.025% carbohydrates, 0.075% agar, 0.5% sodium salts, and 0.02 M phosphate buffer (pH 7.1).

To use the Inocupac system, growth from four or five isolated colonies was picked up with the polypropylene rod attached to the cap. The inoculum rod was replaced into the vial and the inner glass vial was crushed, releasing the broth medium. The entire unit was then mixed in a Vortex mixer, releasing the inoculum from the polypropylene rod. The unit was allowed to incubate for 4 h at 35°C and then compared to a turbidity standard (provided in Inocupac units) which is equivalent in performance to a 0.5 MacFarland standard. If the broth culture was not sufficiently turbid, the unit was reincubated and examined after 6 h and again after 18 to 24 h. Once sufficient turbidity was detected, susceptibility tests were performed. A sterile cotton swab was laid directly onto a Mueller-Hinton agar plate and then saturated with 4 to 6 drops of the broth culture. This was easily accomplished by means of a hole under the seal on the cap of the Inocupac unit. The surface of the agar plate was then inoculated, and disks were applied, as described for the Bauer-Kirby procedure (6, 7).

RESULTS

Inocupac incubation time. The 100 study strains were each subjected to 15 trials with Inocupac units. The ability of the Inocupac units to support growth of different types of microorganisms is summarized in Table 1, which lists the number of trials that could be tested after 4, 6, and 24 h of incubation. Only 1,469 tests were available for analysis because 27 reports failed to record incubation times and one investigator failed to report 4 tests. All of the *Enterobacteriaceae* and *Staphylococcus* spp. could be tested after 4 h. Nearly one-fourth of the *P. aeruginosa* strains required a full 6 h of incubation to reach sufficient turbidity. Most of the enterococci (94%) and 78% of the other streptococci provided adequate growth after 6 h of incubation. Six streptococci failed to grow after 6 h in 29 of 90 trials, and only 6 of those 29 units provided satisfactory tests after overnight incubation. The six streptococci which failed to grow satisfactorily in some Inocupac units represent two enterococci and four other streptococci. Two-thirds of the trials with those six strains provided satisfactory growth after 4 to 6 h. No strain consistently failed to grow in all 15 Inocupac units that were inoculated.

Comparison of zone sizes. Zone diameters recorded with the Bauer-Kirby method were compared directly to those obtained with the Inocupac system (Tables 2 and 3). About 90% of the gram-negative bacilli produced zones which differed by no more than 2 mm. For the purpose of these calculations, a zone was considered to be 6 mm in diameter when there was no inhibition around the 6.35-mm disk. Regression analysis was also used to summarize the data with each antimicrobial drug. Correlation coefficients were calculated for all data and for only those tests with two measurable zones (excluding "no zone" responses). For all drugs, mean differences

TABLE 1. *Time to reach sufficient growth density with the Inocupac system*

Microorganism (no. of trials)	Time (h)[a]			
	4	6	18–24	>24[b]
Escherichia coli (375)	375[c] (100)			
Klebsiella pneumoniae (223)	222 (>99)	1 (<1)		
Proteus mirabilis (178)	175 (98)	3 (2)		
Other *Proteus* spp. (74)	70 (95)	4 (5)		
Providencia spp. (57)	56 (98)	1 (2)		
Serratia spp. (56)	56 (100)			
Pseudomonas aeruginosa (72)	55 (76)	17 (24)		
Other gram-negative (70)	64 (91)	6 (9)		
Staphylococcus spp. (149)	149 (100)			
Enterococcus group (118)	101 (86)	9 (8)	1 (1)	7 (6)[b,d]
Other *Streptococcus* spp. (97)	45 (46)	31 (32)	5 (5)	16 (16)[b,e]

[a] Time needed to reach sufficient turbidity for disk tests.

[b] No growth or growth too light for adequate disk tests.

[c] Expressed as the number (percent) of vials with sufficient turbidity to proceed with disk tests; 15 trials for test strain. Three tests with *Streptococcus mutans* and one with *Streptococcus pneumoniae* were not reported by one investigator.

[d] Growth problems were encountered with 3 of 15 trials with 1 strain of *Streptococcus faecalis* and 4 of 15 trials with 1 strain of *Streptococcus faecium*.

[e] Growth problems were encountered with 4 of 15 trials with 1 strain of a group A *Streptococcus*, 3 of 15 trials with group G *Streptococcus*, 5 of 12 trials with 1 strain of *Streptococcus mutans*, and 4 of 14 trials with 1 strain of *Streptococcus pneumoniae*.

TABLE 2. *Direct comparison of zone diameters obtained with the Inocupac system versus conventional Bauer-Kirby procedure with 75 gram-negative bacilli tested in triplicate by five investigators*

Antimicrobial agent	No. of trials[a]	Difference[b] (mm)									Mean ± standard deviation	Correlation coefficient	
		≤−4	−3	−2	−1	0	+1	+2	+3	≥+4		Including all tests	Excluding "no zone"
Amikacin	1,112	19	13	61	192	387	269	133	26	12	0.2 ± 1.52	0.801	0.688
Ampicillin	921	19	19	43	102	544	112	53	12	17	0.0 ± 1.67	0.973	0.923
Carbenicillin	1,121	31	34	50	142	423	213	136	50	42	0.3 ± 2.06	0.970	0.949
Cephalothin	1,118	25	25	67	124	518	178	103	45	33	0.2 ± 2.24	0.951	0.844
Chloramphenicol	1,120	18	33	66	151	378	234	144	55	41	0.4 ± 2.03	0.938	0.896
Gentamicin	1,121	15	28	56	189	386	278	128	30	11	0.2 ± 1.52	0.872	0.819
Kanamycin	1,122	12	22	45	177	444	270	116	21	15	0.2 ± 1.57	0.953	0.884
Nalidixic acid	1,121	30	18	61	197	370	266	116	49	14	0.1 ± 2.10	0.921	0.852
Nitrofurantoin	1,122	20	15	63	159	455	242	107	39	22	0.2 ± 1.59	0.959	0.937
Tetracycline	1,118	23	13	46	100	568	220	90	41	17	0.2 ± 1.52	0.977	0.943
Trimethoprim-sulfamethoxazole	1,122	36	27	74	149	369	246	122	57	42	0.2 ± 2.02	0.957	0.893
Tobramycin	1,122	27	16	59	172	378	285	129	38	18	0.2 ± 1.94	0.789	0.728
Total number	13,240	275	263	691	1,854	5,220	2,813	1,377	468	284			
Total percent	100	2.1	2.0	5.2	14.0	39.4	21.2	10.4	3.5	2.2			

[a] Total of 1,125 trials should have been recorded with each drug, but some test results were not reported for inclusion in these analyses. The number of tests with ampicillin were decreased because of an early supply problem.

[b] +, Inocupac larger; −, Inocupac smaller. Values are expressed as the number of trials in each category.

TABLE 3. *Direct comparison of zone diameters obtained with Inocupac system versus conventional Bauer-Kirby disk tests*[a]

Antimicrobial agent	No. of trials	Difference[b] (mm)									Mean ± standard deviation	Correlation coefficient	
		≤−4	−3	−2	−1	0	+1	+2	+3	≥+4		Including all tests	Excluding "no zones"
Ampicillin	350	22	13	25	29	79	37	54	29	62	1.0 ± 3.22	0.947	0.939
Cephalothin	350	9	5	25	44	73	71	48	23	52	1.0 ± 2.64	0.937	0.937
Chloramphenicol	350	10	8	17	32	71	48	74	40	51	1.2 ± 2.23	0.876	0.862
Clindamycin	349	8	5	11	28	158	54	33	21	31	0.8 ± 2.52	0.965	0.881
Erythromycin	350	11	8	25	30	98	59	42	31	46	1.0 ± 2.67	0.955	0.933
Gentamicin	349	4	2	20	39	94	70	61	25	34	1.0 ± 2.09	0.926	0.901
Kanamycin	349	3	1	16	39	168	56	37	14	15	0.4 ± 1.66	0.973	0.951
Oxacillin	350	3	5	11	40	201	44	27	9	10	0.2 ± 1.42	0.976	0.868
Penicillin	349	19	12	25	30	89	48	52	23	51	0.8 ± 2.83	0.966	0.955
Tetracycline	349	9	4	23	26	123	51	47	29	37	0.9 ± 2.31	0.971	0.956
Trimethoprim-sulfamethoxazole	350	13	7	12	42	121	55	34	18	48	1.1 ± 4.77	0.849	0.536
Vancomycin	350	3	5	11	32	104	97	51	22	25	0.9 ± 1.71	0.799	0.799
Total number	4,195	114	75	221	411	1,379	690	559	284	462			
Total percent		2.7	1.8	5.3	9.8	32.9	16.4	13.3	6.8	11.0			
Staphylococci	1,787	3.1[c]	3.0	6.7	14.6	32.8	18.7	12.3	4.7	3.9			
Enterococci	1,426	2.1	0.8	4.5	6.5	35.6	15.0	14.4	7.9	13.2			
Other streptococci	982	2.6	1.1	3.9	5.8	28.7	14.4	13.7	8.9	20.9			

[a] Total of 25 gram-positive cocci, each tested in triplicate by five investigators.
[b] +, Inocupac larger; −, Inocupac smaller. Values are expressed as the number of trials in each category.
[c] Expressed as percent of trials in each category.

in zone sizes were less than 0.5 mm and correlation coefficients confirmed a close correlation between methods. Amikacin and tobramycin yielded relatively poor correlation coefficients because the zone measurements tended to cluster within a fairly narrow range.

Tests with the 25 gram-positive cocci demonstrated somewhat larger zones with the Inocupac units, i.e., mean differences were 0.2 to 1.2 mm (Table 3). Most of the major discrepancies in zone sizes involved tests with *Streptococcus* sp., i.e., almost 30% of the Inocupac tests with nonenterococci had zones at least 3 mm larger than those of the Bauer-Kirby tests. With the enterococci, 21% of the Inocupac zones were ≥3 mm larger. Staphylococci tended to produce more nearly comparable zone sizes: 85% of the tests displayed differences no greater than 2 mm.

Intralaboratory variability. Each laboratory performed tests on 3 separate days with both methods. The range of variation between triplicate tests was expressed as the average standard deviation for each group of related microorganisms (Table 4). The precision of the two disk procedures was essentially identical. With both disk methods, the greatest variability was observed with swarming *Proteus* spp. and with the streptococci.

Interlaboratory variability. The mean zone diameters recorded by the five participants were also compared (Table 4). The two testing procedures again demonstrated comparable precision. With most microorganisms, the inter- and intralaboratory precision was nearly comparable. When testing *Proteus mirabilis*, there was considerable variation among investigators. One participant consistently reported *P. mirabilis* zones which were much smaller than those reported by the four other participants. By excluding data reported by that investigator, interlaboratory precision of results with both methods was markedly improved (Table 4). Intralaboratory precision with *P. mirabilis* was also improved by excluding data reported by that investigator.

Quality control data. Three control strains were included with each group of tests. The data reported by all five investigators are summarized in Table 5. With the two testing procedures, mean zone sizes were essentially identical, and the standard deviations suggested similar precision with the two methods.

Interpretive discrepancies. By applying the interpretive zone standards of the National Committee for Clinical Laboratory Standards (7), each zone measurement was classified into a susceptible, intermediate, or resistant category. Interpretive zone standards by Thornsberry et al. (8) were used for gentamicin and tobramycin. The interpretations of the two disk procedures were in complete agreement with 90 to 99.7% of the tests with different antimicrobial agents (Table 6). Major interpretive discrepancies (susceptible with one method but resistant with the other) occurred with 0 to 1.5% of the tests.

For comparative purposes, interpretive agreement between repeated tests with each method was also calculated (Table 6). Each microorganism was tested in triplicate, generating three pairs of results which were free to disagree, i.e., the first and second test, the first and third test, and the second and third test. Complete interpretive agreement was obtained with 91.5 to

TABLE 4. *Intralaboratory and interlaboratory variability in zone diameters[a] with the Inocupac system and conventional Bauer-Kirby method*

Microorganism	Intralaboratory		Interlaboratory	
	Inocupac	Bauer-Kirby	Inocupac	Bauer-Kirby
Staphylococcus	±2.25 (2.12)	±2.48 (2.09)	±1.03 (0.96)	±1.36 (1.51)
Enterococcus	±4.20 (2.93)	±3.20 (2.38)	±1.88 (1.99)	±2.98 (2.86)
Streptococcus, other	±4.18 (2.76)	±4.04 (2.72)	±1.64 (1.94)	±2.60 (2.72)
Escherichia	±2.44 (2.04)	±2.44 (1.91)	±1.61 (1.32)	±1.89 (1.57)
Klebsiella	±2.46 (1.97)	±2.00 (1.97)	±2.30 (1.83)	±2.55 (1.83)
Enterobacter	±1.97 (1.95)	±2.04 (2.00)	±2.94 (2.29)	±3.69 (2.63)
Serratia	±2.59 (2.16)	±2.72 (2.35)	±2.68 (1.68)	±2.60 (1.61)
Proteus mirabilis	±4.15 (2.60)	±4.64 (2.55)	±10.68 (3.50)	±9.95 (3.41)
Proteus, other	±2.68 (2.32)	±2.68 (2.34)	±1.87 (1.63)	±2.43 (1.68)
Providencia	±2.42 (2.09)	±2.56 (2.08)	±2.30 (1.63)	±1.79 (1.74)
Pseudomonas	±1.64 (1.83)	±1.62 (1.81)	±1.69 (1.21)	±1.25 (1.09)
Other gram-negative	±3.02 (2.34)	±3.00 (2.09)	±3.17 (1.86)	±1.91 (1.37)
Total gram-negative	±2.79 (2.37)	±2.85 (2.28)	±4.68 (2.01)	±4.46 (2.04)

[a] Expressed as ±2 standard deviations to represent the 95% confidence limit for a single observation. Numbers in parentheses represent calculations excluding results from one investigator reporting aberrant results with *Proteus mirabilis*.

TABLE 5. *Comparison of zone diameters obtained with the Inocupac system versus conventional Bauer-Kirby procedure with three quality-control strains*

Antimicrobial agent	Zone diameter[a] (mm)					
	E. coli (ATCC 25922)		*P. aeruginosa* (ATCC 27853)		*S. aureus* (ATCC 25923)	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Amikacin	19.0/18.8	1.00/0.92	17.7/17.1	0.85/0.88	—	—
Ampicillin	18.6/18.3	1.34/1.14	NZ/NZ[c]	NZ/NZ	31.0/30.6	1.88/1.49
Carbenicillin	24.6/24.4	1.37/1.15	19.7/19.3	1.19/0.98	—	—
Cephalothin	19.6/19.2	1.13/1.30	NZ/NZ	NZ/NZ	33.0/32.9	1.40/1.58
Chloramphenicol	23.5/23.1	1.48/1.05	NZ/NZ	NZ/NZ	23.7/23.6	1.42/1.59
Clindamycin	—[b]	—	—	—	26.2/26.0	1.22/1.16
Erythromycin	—	—	—	—	26.8/26.5	1.14/1.56
Gentamicin	21.9/21.7	0.99/0.86	18.3/17.9	1.01/0.81	23.1/22.9	1.37/1.21
Kanamycin	21.6/21.3	1.20/0.98	NZ/NZ	NZ/NZ	22.6/22.1	1.28/1.02
Nalidixic acid	23.9/23.4	1.38/1.22	NZ/NZ	NZ/NZ	—	—
Nitrofurantoin	21.8/21.2	1.23/1.50	NZ/NZ	NZ/NZ	—	—
Oxacillin	—	—	—	—	20.9/20.8	0.89/1.19
Penicillin	—	—	—	—	31.7/31.1	1.75/1.71
Tetracycline	23.0/22.7	1.44/1.40	12.2/11.6	1.35/1.27	27.6/27.4	1.62/1.58
Tobramycin	20.8/20.5	1.30/1.13	22.2/21.5	0.98/1.11	—	—
Trimethoprim-sulfamethoxazole	26.6/26.2	1.37/1.00	NZ/NZ	NZ/NZ	28.6/28.5	1.96/2.17
Vancomycin	—	—	—	—	17.7/17.5	0.92/0.80

[a] Based on 74 tests with *Escherichia coli* and *Pseudomonas aeruginosa* and 42 tests with *Staphylococcus aureus*. Inocupac system/Bauer-Kirby method.

[b] —, This antimicrobial agent was not tested with this organism.

[c] NZ/NZ, No zone of inhibition present for either system.

TABLE 6. *Interpretive agreement between the Inocupac system and Bauer-Kirby procedure*[a]

Antimicrobial agent (no. of trials)	% Agreement (% major discrepancies)		
	Inoc vs. B-K[b]	Within Inoc tests[c]	Within B-K tests[c]
Amikacin (1,112)	96.1 (0.6)	96.6 (0.5)	95.7 (0.8)
Ampicillin (1,271)	96.5 (0.6)	96.2 (1.2)	96.0 (1.3)
Carbenicillin (1,120)	92.5 (0.2)	92.6 (0.3)	93.1 (0.4)
Cephalothin (1,468)	91.4 (0.8)	93.7 (1.1)	91.5 (1.1)
Chloramphenicol (1,470)	94.5 (0.5)	94.7 (0.5)	93.7 (0.5)
Clindamycin (349)	98.0 (0.6)	99.4 (0.0)	97.8 (0.5)
Erythromycin (350)	90.0 (0.9)	91.7 (1.2)	91.8 (0.0)
Gentamicin (1,470)	94.4 (0.5)	95.7 (0.8)	93.3 (0.7)
Kanamycin (1,471)	94.8 (0.4)	94.5 (0.8)	93.7 (0.7)
Nalidixic acid (1,121)	91.7 (0.8)	89.8 (0.9)	92.0 (0.2)
Nitrofurantoin (1,122)	93.9 (0.5)	92.5 (0.9)	92.2 (0.6)
Oxacillin (350)	99.7 (0.0)	100.0 (0.0)	98.9 (0.0)
Penicillin (349)	91.4 (0.0)	95.3 (0.0)	94.5 (0.0)
Tetracycline (1,467)	94.2 (0.2)	94.5 (0.4)	93.6 (0.7)
Trimethoprim-sulfamethoxazole (1,472)	97.5 (1.2)	97.0 (1.5)	97.0 (1.4)
Tobramycin (1,122)	97.5 (1.5)	97.0 (1.6)	97.1 (1.8)
Vancomycin (350)	99.7 (0.0)	100.0 (0.0)	99.5 (0.0)

[a] A total of 100 microorganisms were tested in triplicate by each of five investigators. Inoc, Inocupac; B-K, Bauer-Kirby.

[b] Compares the susceptibility results for 15 pairs of trials on isolates for which the susceptibility test was completed.

[c] Compares the susceptibility results of three trials on each isolate for the respective inoculum standardizing system.

100% of the Bauer-Kirby tests with different antimicrobial agents. Similar reproducibility was obtained with the Inocupac system. Furthermore, discrepancies between methods was about the same order of magnitude as those obtained when either method was repeated on 3 separate days.

Table 7 summarizes the same type of interpretive comparisons, according to the type of microorganism being tested. Although the nonenterococcal streptococci showed considerable variation in zone sizes, the interpretations were rarely affected. Since the enterococci tended to produce somewhat larger zones with the Inocupac system, minor interpretive discrepancies occurred with about 10% of the tests, but major interpretive discrepancies involved only 1% of the tests. Difficulties in measuring zones with *P. mirabilis* resulted in only 2.9 to 3.3% major interpretive discrepancies with either method of preparing the inoculum.

DISCUSSION

The present study provides an excellent opportunity to document the inter- and intralaboratory precision of the Bauer-Kirby technique. With most microorganisms, a 95% confidence

TABLE 7. *Interpretative agreement between the Inocupac system and the conventional Bauer-Kirby procedure by microorganism*

Microorganism (no. of trials)	% Agreement (% major discrepancies)		
	Inoc vs. B-K[a]	Within Inoc tests[b]	Within B-K tests[b]
Escherichia coli (4,296)	96.4 (0.4)	97.2 (0.4)	96.3 (0.6)
Klebsiella pneumoniae (2,657)	94.0 (0.3)	93.4 (0.5)	93.9 (0.1)
Proteus mirabilis (2,157)	92.9 (2.1)	92.8 (2.9)	90.4 (3.3)
Proteus, other sp. (900)	95.7 (0.6)	95.9 (0.8)	94.2 (0.7)
Providencia sp. (716)	92.6 (0.6)	90.5 (0.8)	90.6 (0.4)
Serratia sp. (717)	94.4 (0.1)	93.1 (0.8)	92.7 (1.4)
Pseudomonas aeruginosa (900)	93.0 (0.0)	93.1 (0.0)	95.6 (0.0)
Enterobacter sp. (360)	94.7 (0.0)	93.6 (0.3)	92.2 (0.0)
Other gram-negative (537)	94.8 (0.2)	95.7 (0.2)	96.3 (0.0)
Staphylococcus sp. (1,787)	99.0 (0.0)	99.5 (0.0)	99.1 (0.0)
Enterococcus (1,426)	88.8 (1.3)	90.3 (1.6)	89.8 (1.0)
Other *Streptococcus* sp. (982)	96.2 (0.7)	97.1 (0.7)	96.1 (0.5)

[a] Compares the susceptibility results for 15 pairs of trials on isolates for which the susceptibility test was completed. Inoc, Inocupac; B-K, Bauer-Kirby.

[b] Compares the susceptibility results of three trials on each isolate for the respective inoculum standardizing system.

limit of ±2 or 3 mm may be assumed for a single observation, i.e., repeated tests might be expected to vary over a range of 4 to 6 mm. With some streptococci, a range of variability of 6 to 8 mm might be expected. Reproducible results are much more difficult to obtain with swarming *Proteus* spp. because the zone edges are often poorly defined and difficult to measure consistently. The agar-overlay method (3) provides much clearer zones of inhibition with such microorganisms (2). Tests with two or three quality control strains do not adequately assess the precision of a testing procedure; a wide variety of microorganisms such as those included in the present study should be tested to properly estimate precision.

Quantitative dilution tests are usually accepted as reference methods for evaluating disk susceptibility tests. Dilution tests are generally considered to be adequately controlled if repeated tests vary no more than ±1 $\log_2$ dilution interval (range of two dilution steps). With most antimicrobial agents, that magnitude of variability is generally equivalent to a range of 6 to 8 mm in zone diameters (5). Consequently, the precision of both disk procedures, reported in this study, may be considered quite acceptable.

Although the zones of inhibition may vary somewhat, the interpretations of most disk tests are not affected. With those strains giving mean zones near the interpretive breakpoints, minor differences in zone sizes could change the interpretation from susceptible to intermediate or from resistant to intermediate. Less than 1% of our tests varied from resistant to susceptible, and less than 10% varied from susceptible or resistant to intermediate. Interpretive variability is more significant with certain drug-microorganism combinations, i.e., enterococci vs. penicillin (16% were susceptible with Inocupac but intermediate with the Bauer-Kirby method).

When the inoculum was prepared in an Inocupac unit, the results were essentially the same as those obtained with the standardized Bauer-Kirby method. Difficulties were encountered with certain streptococci, since the Inocupac media failed to consistently support growth of some strains. Furthermore, exceptionally large zones of inhibition were observed with some streptococci, possibly because the Inocupac units occasionally provided an inoculum which was too light. Entirely satisfactory results were obtained with the gram-negative bacilli and staphylococci: in clinical practice, those are the types of microorganisms for which disk tests are most appropriate. On the rare occasion when other types of microorganisms are to be tested, agar or broth dilution tests are generally preferred (1). Disk diffusion procedures are not appropriate for testing microorganisms with prolonged growth rates, i.e., some streptococci, many anaerobes, etc.

The Inocupac system offers a convenient, simple alternative to the standardized Bauer-Kirby method. Its major advantage is that it eliminates the need to adjust turbidity of broth cultures, a time-consuming chore which is frequently done poorly in many clinical laboratories. The participants in the present study are experienced investigators, and all tests were performed with extreme care. It would be interesting to determine what type of precision would be observed if a much broader representative sample of clinical laboratories could have been included in such an evaluation. One might anticipate somewhat more reproducible results with the Inocupac system than with the Bauer-Kirby method in such laboratories, because the subjective step of turbidity adjustment has been eliminated. Microbiologists who are experiencing difficulties in maintaining satisfactory control of the Bauer-Kirby procedure might consider utilization of the Inocupac system.

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