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Comparison of Spiral Gradient and Conventional Agar Dilution for Susceptibility Testing of Anaerobic Bacteria

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Antimicrobial susceptibility tests were performed on brucella laked blood agar with 340 isolates and 14 antimicrobial agents by the standard agar dilution technique and the spiral gradient technique in which antibiotic concentrations were established by diffusion from the agar surface. For comparison, spiral gradient MICs were determined by calculating antimicrobial concentrations at growth endpoints and rounding up to the next twofold incremental concentration. The cumulative percentage of strains susceptible at the breakpoint determined from spiral gradient data was within 10%, generally, of the percentage of strains susceptible at the breakpoint determined from agar dilution data. The overall agreement between the two techniques (within one doubling dilution) was 90.6%. The spiral gradient agar dilution technique is a reasonable alternative to the conventional agar dilution technique for susceptibility testing of anaerobic bacteria.

The necessity of performing susceptibility tests on anaerobic isolates has been reviewed recently (3, 14). While it is generally agreed that anaerobes do not need to be tested routinely, there are cases in which such testing is required. Also, local antibiograms need to be monitored and activities of new antimicrobial agents need to be determined. Thus, the need for reliable methods for susceptibility testing of anaerobic bacteria extends from small hospital laboratories to large research centers. The National Committee for Clinical Laboratory Standards (NCCLS) has published recommendations on testing (9); these include agar dilution (AD) methods and broth micro- and macrodilution methods. Broth disk elution, the most commonly used technique (7), is no longer approved by the NCCLS. This leaves fewer options open to clinical laboratories for testing anaerobic isolates.

The spiral plater (Spiral System Instruments, Bethesda, Md.) has been used for bacterial enumeration for some time (2). The spiral gradient endpoint (SGE) system for determining MICs has been introduced recently (4, 10). Hill and Schalkowsky (4) found an overall 90% agreement between the SGE and standard AD methods for gram-negative anaerobic bacilli. The spiral plater deposits a set amount of antimicrobial stock solution in a spiral pattern on an agar plate, resulting in a radially decreasing concentration gradient. Strains of bacteria are inoculated onto the plate in a radial fashion, and the MIC can be determined from the radius at which growth stops. The antibiotic concentration gradient on a plate typically spans eight twofold dilutions, thus cutting down considerably on the time and materials needed to perform the test.

This report summarizes the data collected during a number of in vitro studies in which the SGE system was tested in parallel with a conventional incremental AD test in our laboratory and suggests that the SGE test gives results that are at least equivalent to those of the conventional method for the organisms and antimicrobial agents tested.

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(Portions of this study were presented at the 90th Annual Meeting of the American Society for Microbiology [8].)

MATERIALS AND METHODS

The species of anaerobic organisms tested in this study are listed in Tables 1 and 2. All bacteria were randomly selected recent clinical isolates from the Wadsworth Anaerobe Laboratories (Veterans Administration Wadsworth Medical Center, Los Angeles, Calif.). Bacteria were identified by established procedures (5, 13). The conventional incremental AD technique was done as described previously (13) by using an inoculum of 10^5 CFU and brucella laked blood agar. Plates were incubated in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) or in an anaerobic chamber (Anaerobe Systems, San Jose, Calif.) for 48 h at 37°C. AD MICs were defined as the lowest concentration of antimicrobial agent permitting no growth, one discrete colony, or a barely visible haze. When a distinct change from the growth control was observed followed by a persistent light growth (haze), the concentration at which the first change occurred was noted, as was the concentration at which the haze ended. Reference strains of *Bacteroides fragilis* (ATCC 25285) and *Bacteroides thetaiotaomicron* (ATCC 29741) were used as controls in each test.

Antimicrobial agents were obtained as powders from their respective manufacturers, as follows: chloramphenicol and sparfloxacin, Parke, Davis & Co. (Morris Plains, N.J.); ciprofloxacin, Miles Laboratories (Elkhart, Ind.); cefoxitin and imipenem, Merck Sharp & Dohme (Rahway, N.J.); metronidazole, Searle Laboratories (Chicago, Ill.); Win 57273, Sterling Winthrop (Rensselaer, N.J.); clindamycin, Upjohn (Kalamazoo, Mich.); ampicillin, cefoperazone, and sulbactam, Pfizer Laboratories (New York, N.Y.); cefotaxime, Hoechst-Roussel (Somerville, N.J.); cefotetan, Stuart Pharmaceuticals (Wilmington, Del.); ceftizoxime, Fujisawa (Philadelphia, Pa.); and ceftriaxone, Roche Laboratories (Nutley, N.J.).

The SGE test relies on creation of a radial gradient of antibiotic concentrations in the agar of a prepoured petri dish. The spiral plater (Fig. 1) deposits a stock solution of the

TABLE 1. Strains tested with penicillin and cephalosporin agents

Organism	No. of strains tested[a]						
	Ampicillin-sulbactam (2:1)	Cefotaxime	Cefotetan	Cefoxitin	Ceftizoxime	Ceftizoxime-sulbactam (2:1)	Ceftriaxone
Bacteroides fragilis	48	38	33	38	50	52	38
Other species of the *Bacteroides fragilis* group	60	61	58	61	56	61	61
Bacteroides gracilis	NT[b]	6	6	6	6	NT	6
Pigmenting *Bacteroides* species[c]	19	18	18	18	19	19	18
Other *Bacteroides* species	19	14	14	14	19	19	14
Fusobacterium species	21	30	29	31	23	23	31
Peptostreptococcus species	16	14	14	14	15	16	13
Clostridium species	18	17	17	17	17	19	17
Gram-positive rods[d]	17	19	18	19	19	19	19
Total	218	217	207	218	224	228	217

[a] Stock concentrations of antimicrobial agents were as follows: ampicillin-sulbactam, 10,300 μg/ml and 5,150 μg/ml; cefotaxime, 40,000 μg/ml; cefotetan, 40,000 μg/ml; cefoxitin, 40,000 μg/ml; ceftizoxime, 40,000 μg/ml; ceftizoxime-sulbactam, 25,800 μg/ml and 12,900 μg/ml; ceftriaxone, 40,000 μg/ml.
[b] NT, not tested.
[c] Includes two *Porphyromonas* strains.
[d] Includes *Eubacterium*, *Lactobacillus*, *Propionibacterium*, and *Actinomyces* species.

antibiotic at decreasing rates along a spiral pattern on the surface of the agar. Figure 2 illustrates a spiral gradient (SG) formed with crystal violet dye.

The SGEs were measured in accordance with the instructions provided in the user guide (12). Spiral gradients were made on brucella agar (Difco, Detroit, Mich.) with 5% laked sheep blood and vitamin K_1 (10 μg/ml) either prepared fresh or available commercially (Anaerobe Systems). Antimicrobial agents and the stock concentrations used are listed in Tables 1 and 2. A total of 55 to 60 ml of medium was dispensed into a 150-mm-diameter plate, giving an agar depth of approximately 3.8 mm. Antimicrobial stock solutions were deposited on the plates by using a model DU spiral plater (Spiral System Instruments). After stock solutions were deposited onto the plates, the plates were placed into an anaerobic chamber for 4 to 6 h before they were inoculated with bacteria. Control SG plates were prepared by depositing sterile deionized water onto the plates.

Test strains were inoculated onto the surface of the agar by using the radial replicator (Fig. 3), which is similar in concept to the familiar Steers replicator. The inoculating head (in this case, 15 radial sets of hollow pins arranged in "spokes") incorporates the inoculum from a plate containing the various strains in corresponding troughs; the bottom plate then slides over so that the inoculating head can inoculate the agar plate. This inoculator is automated and standardizes the amount of time the head spends in the troughs and on the agar plate, thus minimizing some possible variation in inoculum. The same 0.5 McFarland standard suspension that was used in the troughs was used in the wells of a Steers replicator to inoculate the AD plates. Plates were incubated for 48 h at 35 to 37°C either in an anaerobic chamber (Anaerobe Systems) or anaerobic jars.

SGEs were measured as the radius from the center of the plate to the endpoint of growth (in millimeters) by using a clear plastic template (Spiral System Instruments) (Fig. 4). The various designations of growth endpoints are illustrated in Fig. 5. The radius at which growth ended was termed the "tail-ending" radius. At times, a heavy, confluent line of growth did not end abruptly but became much less dense (similar to the haze seen on agar dilution plates). When this occurred, a second "tail-beginning" radius was measured at the point where the nature of the growth changed. Occasionally, isolated colonies that persisted beyond the tail were seen; these colonies were referred to as "outliers," and the radius at which the presence of these colonies was no longer

TABLE 2. Strains tested with other antimicrobial agents

Organism	No. of strains tested[a]						
	Chloramphenicol	Ciprofloxacin	Imipenem	Metronidazole	Win 57273	Clindamycin	Sparfloxacin
Bacteroides fragilis	12	12	7	12	12	56	49
Other species of the *Bacteroides fragilis* group	19	19	11	19	19	62	71
Bacteroides gracilis	NT[b]	NT	NT	NT	NT	7	8
Pigmenting *Bacteroides* species[c]	NT	NT	NT	NT	NT	13	14
Other *Bacteroides* species	NT	NT	NT	NT	NT	21	21
Fusobacterium species	10	10	9	10	10	32	32
Clostridium species	11	11	11	11	11	24	24
Gram-positive rods[d]	13	13	10	13	13	19	19
Peptostreptococcus species	NT	NT	NT	NT	NT	20	20
Total	65	65	48	65	65	254	265

[a] Stock concentrations of antimicrobial agents were as follows: chloramphenicol, 5,000 μg/ml; ciprofloxacin, 5,200 μg/ml; imipenem, 4,300 μg/ml; metronidazole, 4,200 μg/ml; Win 57273, 4,500 μg/ml; clindamycin, 4,600 μg/ml; sparfloxacin, 4,000 μg/ml.
[b] NT, not tested.
[c] Includes two *Porphyromonas* strains.
[d] Includes *Eubacterium*, *Lactobacillus*, *Propionibacterium*, and *Actinomyces* species.

FIG. 1. Spiral plater. Photograph courtesy of Spiral System Instruments.

seen was the outlier radius. The outlier radius was not measured or used in the calculations in this study.

The data were entered into a computer software program provided by the manufacturer that determines the concentration of drug on the basis of the radius of growth and the molecular weight (i.e., diffusion characteristics) of the antimicrobial agent. For comparison with conventional incremental AD values, tail-ending concentrations (TECs) were rounded to the next highest twofold concentration to determine a SG MIC. The program provided an option to use tail-beginning concentrations (TBCs) (i.e., the point at which growth changed from opaque, heavy growth to either a haze or a series of individual colonies) as endpoints for analyses, and these endpoints were used when they were consistent with NCCLS recommendations (9) for reading substantial reductions, barely visible hazes, or both. Earlier studies in our laboratory described hazes seen with some species of *Fusobacterium* and beta-lactam agents; for those strains, we found that the haze represented cell wall-deficient forms of the *Fusobacterium* strains (6). The problem exists, to some extent, with most gram-negative anaerobic bacteria and some beta-lactam agents. Ceftizoxime, for example, is a drug likely to cause this haze.

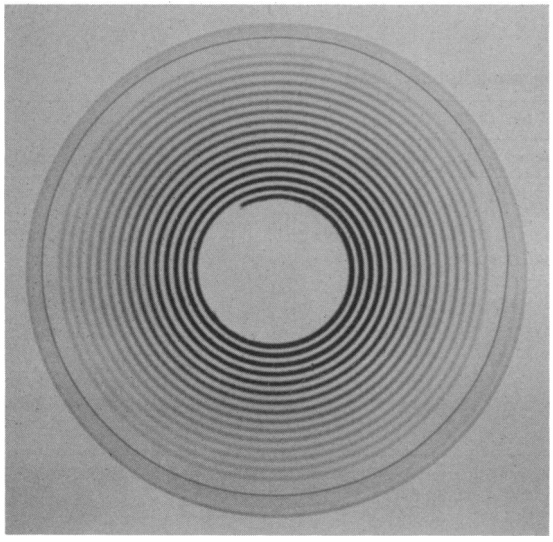

FIG. 2. Spiral gradient of crystal violet dye.

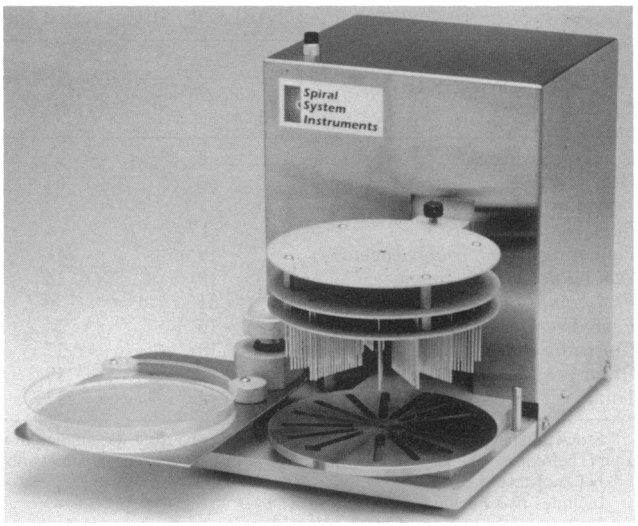

FIG. 3. Radial replicator.

For estimating variability in the SGE technique, the two control strains (ATCC 25285 and ATCC 29741) were tested in duplicate by three different technicians. Each technician prepared a separate 0.5 McFarland standard and separate antimicrobial stock solutions, and each strain was replicated on each plate seven times. Mean TECs and coefficients of variation (the ratio of the standard deviation to the mean) were calculated for chloramphenicol, ceftizoxime, ciprofloxacin, and metronidazole.

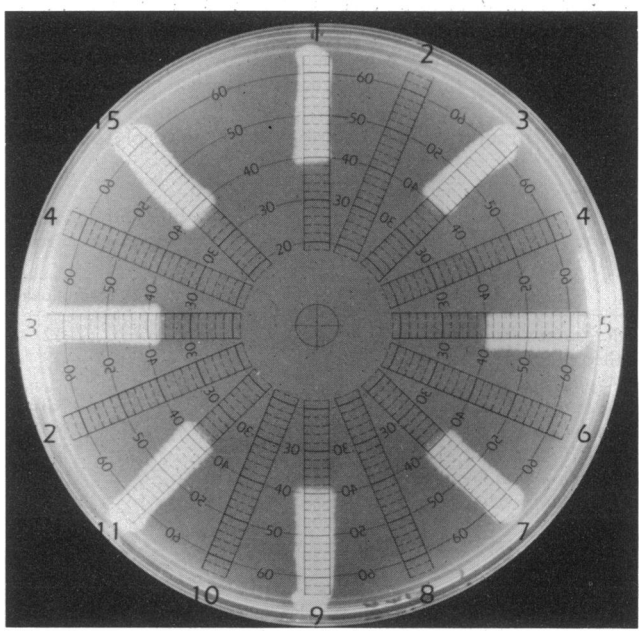

FIG. 4. *E. coli* deposited with a replicator onto an ampicillin gradient. The view is through the SGE template for measurement of the endpoint location.

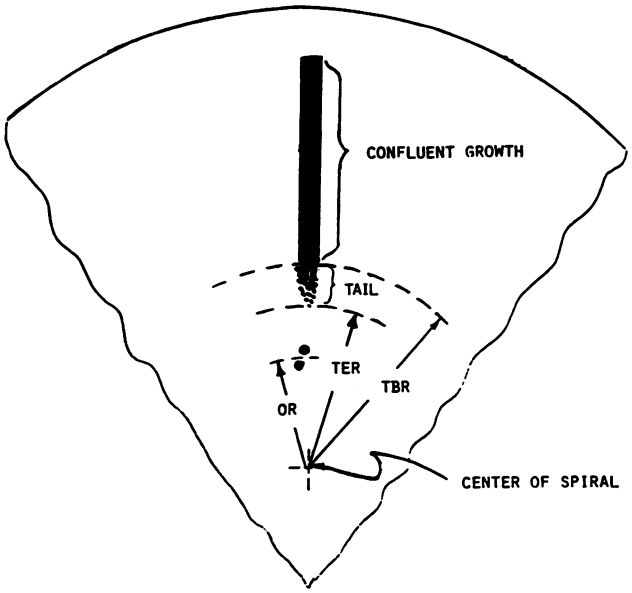

FIG. 5. Line representation of SGE endpoint measurements. TBR, tail-beginning radius; TER, tail-ending radius; OR, outlier radius.

RESULTS

Tables 3 and 4 list the cumulative percentages of strains susceptible to beta-lactam agents and other antimicrobial agents, respectively, for each group of organisms. In most cases, results were essentially the same by both techniques. The greatest discrepancies in the percentage of susceptible strains at the breakpoint between the two methods occurred for *Clostridium* species with cefoxitin (23%; Table 3) and for *Fusobacterium* species with ciprofloxacin (30%; Table 4). However, TECs for individual strains in these two instances were near the critical AD concentration. For example, for three clostridia TECs were between 30 and 32 μg/ml, and hence, SG MICs of 32 μg/ml (and therefore termed susceptible, since the breakpoint for cefoxitin is 32 μg/ml), whereas their AD MICs were 64 μg/ml (resistant). For the nine *Fusobacterium* strains that were compared by the two techniques, MICs for two strains (or 22%) were 2 μg/ml by the SGE technique and 4 μg/ml (and thus resistant) by the conventional agar technique. In general, discrepancies between the two methods could be attributed to one of the following two causes: (i) endpoints were difficult to read on one or both tests, or (ii) MICs were close to breakpoint concentrations (and thus, MICs for the organisms determined by the two techniques could have been within one twofold dilution of each other, yet one test resulted in a susceptible designation and one resulted in a resistant designation).

Agreement between the SG and AD techniques was also compared on the basis of the number of doubling dilutions by which their MICs differed (Tables 5 and 6). In cases in which MICs obtained by both methods were within the range of dilutions (1,238 measurements), agreement was 90.6% overall; within two dilutions, agreement was usually 100%. Again, the discrepancies generally occurred with those organism-drug combinations that resulted in tailing endpoints. In some cases, the MICs were not within the range of values tested in either system (off-scale or off-boundary measurements); if the two measurements with off-boundary values in both systems were in the same direction, it was considered off-boundary agreement (indicated in Tables 5 and 6 by OB). If one measurement had a discrete value (e.g., 256 μg/ml) and the other was off boundary (e.g., >256 μg/ml), they were not considered to be in agreement and therefore the percent OB agreement can be lower than that obtained when the actual measurements were compared.

In the case of sparfloxacin, we noticed a greater discrepancy between AD and SG results than was seen with other drugs; several of the discrepant strains were retested by using small AD increments to reproduce the concentrations for every 2 mm on the SGE plate. The results obtained by the two techniques and compared in this manner were nearly identical.

SGE reproducibility was assessed by having three different individuals perform the test on the two control strains (Table 7). Overall, the coefficient of variation ranged from 15 to 32%, which is considerably better than the ±one twofold dilution error allowed by the agar dilution technique.

DISCUSSION

The NCCLS has recently published a revised approved standard for susceptibility testing of anaerobic bacteria (9). The methods include AD, broth microdilution, and broth macrodilution tests. AD testing, which is used in many large research centers, is too costly and labor intensive for many clinical laboratories. Although the broth microdilution test is more convenient, some of the more fastidious anaerobes do not grow sufficiently well in this system to be tested with it. Also, the choice of antibiotics is limited by the panels produced commercially (unless a laboratory can produce their own plates) and may not be reflective of the hospital formulary. The void created in anaerobic susceptibility testing by the disapproval of the broth disk elution system emphasizes the need for the development of more convenient and reliable techniques that will allow clinical laboratories to determine antibiograms in a reliable, cost-effective manner.

Use of the SGE method depends on its providing reliable measurements of antibiotic concentrations at any location along the gradient, allowing for the fact that diffusion alters concentrations with time. This point is of particular importance with anaerobic bacteria, which generally grow more slowly than aerobic organisms. Relevant diffusion rates were initially studied by Schalkowsky et al. (11) in the course of the development of the SGE method by using spectrophotometric readings of agar plugs following deposition of a methotrexate gradient, and confirming data were presented by Cody et al. (1). The correction for the diffusion in the computation of the drug concentration was incorporated into the methodology after studies by Hill and Schalkowsky (4) and was found to be effective for antimicrobial agents spanning a wide range of molecular weights. This correction is critical to the application of the SGE method to anaerobes. The correlation between the AD and SGE methods seen in this study is a validation of those diffusion computation corrections.

The SGE technique addresses another deficiency in the conventional AD method. The standard allowable error for the AD method is considered to be ±one twofold dilution (±100%), and this has been confirmed in studies in our laboratory (15). When values cluster near the breakpoint (e.g., in the case of the *B. fragilis* group and beta-lactam antibiotics), an organism termed susceptible on one occasion may be retested and termed resistant. Clustering around the

TABLE 3. Cumulative percent susceptible at a selected range of concentrations

Organism	Method	% Susceptible[a]						
		Ampicillin-sulbactam (2:1)	Cefotaxime	Cefotetan	Cefoxitin	Ceftizoxime[b]	Ceftizoxime-sulbactam (2:1)[b]	Ceftriaxone
Bacteroides fragilis	SG	98, 100, 100	3, 21, 61	64, 85, 85	82, 87, 95	10, 18, 72, 94	94, 98, 100, 100	5, 24, 50
	AD	98, 100, 100	5, 26, 68	67, 79, 79	81, 91, 97	14, 38, 82, 96	100, 100, 100, 100	11, 32, 68
Other species of the *Bacteroides* fragilis group	SG	92, 100, 100	25, 44, 67	16, 26, 34	39, 74, 95	34, 45, 59, 86	87, 100, 100, 100	20, 43, 60
	AD	90, 97, 100	23, 43, 70	19, 31, 38	28, 75, 94	27, 48, 63, 89	97, 100, 100, 100	22, 42, 60
Bacteroides gracilis	SG	NT[c]	17, 17, 17	17, 17, 17	17, 17, 17	17, 17, 17, 17	NT	17, 17, 17
	AD	NT	0, 0, 0	0, 0, 0	33, 33, 33	33, 33, 33, 33	NT	0, 0, 0
Pigmenting *Bacteroides* species	SG	100, 100, 100	100, 100, 100	94, 100, 100	100, 100, 100	100, 100, 100, 100	100, 100, 100, 100	94, 94, 100
	AD	100, 100, 100	100, 100, 100	94, 100, 100	100, 100, 100	100, 100, 100, 100	100, 100, 100, 100	89, 94, 100
Other *Bacteroides* species	SG	100, 100, 100	86, 86, 100	86, 86, 93	71, 86, 86	89, 95, 100, 100	95, 100, 100, 100	71, 86, 93
	AD	100, 100, 100	69, 92, 100	93, 93, 100	85, 100, 100	89, 89, 100, 100	100, 100, 100, 100	77, 92, 92
Fusobacterium species[d]	SG	100, 100, 100	77, 83, 83	100, 100, 100	97, 97, 97	83, 83, 83, 83	83, 83, 83, 83	87, 90, 90
	AD	95, 95, 100	78, 85, 85	100, 100, 100	100, 100, 100	78, 83, 83, 83	86, 86, 86, 91	83, 86, 86
Peptostreptococcus species	SG	100, 100, 100	100, 100, 100	100, 100, 100	100, 100, 100	100, 100, 100, 100	100, 100, 100, 100	100, 100, 100
	AD	100, 100, 100	100, 100, 100	100, 100, 100	100, 100, 100	100, 100, 100, 100	100, 100, 100, 100	100, 100, 100
Clostridium species	SG	94, 100, 100	82, 82, 82	59, 65, 76	59, 76, 88	53, 76, 82, 82	63, 84, 89, 89	82, 88, 94
	AD	94, 100, 100	80, 80, 87	50, 63, 75	53, 53, 87	44, 81, 81, 88	56, 72, 89, 89	87, 87, 87
Gram-positive rods	SG	100, 100, 100	84, 89, 100	83, 89, 94	95, 95, 95	79, 84, 95, 95	84, 95, 100, 100	84, 89, 95
	AD	100, 100, 100	84, 84, 95	83, 89, 94	95, 95, 95	84, 95, 95, 95	94, 94, 100, 100	89, 89, 95

[a] Values are percent susceptible at one dilution below breakpoint, at the breakpoint, and one dilution above the breakpoint. Antimicrobial agent breakpoints were as follows: ampicillin-sulbactam, 16 μg/ml; cefotaxime, 32 μg/ml; cefotetan, 32 μg/ml; cefoxitin, 32 μg/ml; ceftizoxime and ceftizoxime-sulbactam, 32 μg/ml; ceftriaxone, 32 μg/ml.

[b] The NCCLS has recommended a breakpoint of 64 μg/ml for ceftizoxime with agar dilution (10). The four cumulative percent susceptible values listed are for 16, 32, 64, and 128 μg/ml, respectively.

[c] NT, not tested.

[d] The spiral gradient cumulative percent susceptible for *Fusobacterium* species is based on tail-beginning concentrations. This conforms to the NCCLS recommendation of determining MIC endpoints when there is a substantial reduction of growth, a barely visible haze, or both (9).

TABLE 4. Cumulative percent susceptible at a selected range of concentrations

Organism	Method	% Susceptible[a]						
		Chloramphenicol	Ciprofloxacin	Imipenem	Metronidazole	Win 57273	Clindamycin	Sparfloxacin
Bacteroides fragilis	SG	100, 100, 100	0, 0, 8	100, 100, 100	83, 100, 100	92, 100, 100	55, 77, 86	16, 84, 100
	AD	83, 100, 100	0, 0, 20	100, 100, 100	100, 100, 100	82, 100, 100	30, 66, 88	24, 67, 98
Other species of the *Bacteroides fragilis* group	SG	68, 95, 100	11, 16, 21	100, 100, 100	100, 100, 100	89, 100, 100	39, 50, 79	10, 55, 96
	AD	32, 100, 100	6, 6, 22	100, 100, 100	100, 100, 100	95, 100, 100	29, 47, 69	4, 61, 94
Bacteroides gracilis	SG	NT[b]	NT	NT	NT	NT	57, 57, 71	75, 75, 75
	AD						57, 57, 57	75, 75, 75
Pigmenting *Bacteroides* species	SG	NT	NT	NT	NT	NT	100, 100, 100	7, 86, 100
	AD						100, 100, 100	0, 69, 100
Other *Bacteroides* species	SG	NT	NT	NT	NT	NT	90, 90, 90	76, 81, 100
	AD						90, 90, 90	30, 75, 100
Fusobacterium species[c]	SG	100, 100, 100	10, 30, 90	100, 100, 100	100, 100, 100	80, 90, 100	84, 88, 97	41, 69, 84
	AD	100, 100, 100	0, 0, 67	100, 100, 100	100, 100, 100	80, 80, 100	88, 88, 94	16, 66, 78
Clostridium species	SG	64, 100, 100	9, 9, 27	91, 100, 100	91, 91, 91	100, 100, 100	29, 38, 58	50, 58, 75
	AD	73, 100, 100	9, 9, 9	73, 100, 100	91, 91, 91	100, 100, 100	12, 21, 50	50, 54, 67
Gram-positive rods	SG	100, 100, 100	62, 62, 77	100, 100, 100	62, 62, 62	92, 92, 92	89, 95, 95	79, 84, 89
	AD	92, 100, 100	10, 50, 70	100, 100, 100	54, 62, 62	92, 92, 92	79, 95, 95	58, 74, 84
Peptostreptococcus species	SG	NT	NT	NT	NT	NT	60, 75, 75	85, 90, 90
	AD						55, 70, 75	84, 89, 95

[a] Values are percent susceptible at one dilution below breakpoint, at the breakpoint, and one dilution above the breakpoint. Antimicrobial agent breakpoints were as follows: chloramphenicol, 16 μg/ml; ciprofloxacin, 2 μg/ml; imipenem, 8 μg/ml; metronidazole, 16 μg/ml; Win 57273, 2 μg/ml; clindamycin, 2 μg/ml; sparfloxacin, 2 μg/ml.

[b] NT, not tested.

[c] The spiral gradient cumulative percent susceptible for *Fusobacterium* species is based on tail-beginning concentrations. This conforms to the NCCLS recommendation of determining MIC endpoints when there is a substantial reduction of growth, a barely visible haze, or both (9).

breakpoint is a characteristic of the organism-drug interaction; the greater sensitivity of the SGE test in determining MICs (±20%) because of a continuous concentration gradient reduces the numbers of strains that may be variably labeled susceptible or resistant. For example, in this study, 99 strains of the *B. fragilis* group were tested with cefoxitin. AD MICs for 69 strains (70%) were within one dilution of the 32-μg/ml breakpoint and, thus, could be termed as variably susceptible or resistant. If one assumes a 20% variability for the SGE test (i.e., ±6.4 μg/ml), MICs for 18 strains (19%) were within this breakpoint range. Because the AD test is inherently more variable than the SGE technique, it can be

TABLE 5. Agreement between SG and AD MICs

Organism	% Agreement[a]						
	Ampicillin-sulbactam (2:1)	Cefotaxime	Cefotetan	Cefoxitin	Ceftizoxime	Ceftizoxime-sulbactam (2:1)	Ceftriaxone
Bacteroides fragilis	98, 100 (46)	94, 100 (34)	90, 97, 100 (31)	94, 97, 100 (37)	86, 90, 100 (49)	74, 80, 100 (50)	94, 100 (33)
Other species of the *Bacteroides fragilis* group	98, 100 (57)	95, 100 (58)	84, 95, 100 (38)	93, 100 (58)	94, 96, 100 (52)	81, 87, 100 (54)	96, 98, 100 (53)
Pigmenting *Bacteroides* species	OB 88 (16)	OB 100 (14)	OB 100 (12)	OB 100 (15)	OB 94 (17)	OB 100 (17)	OB 100 (14)
Other *Bacteroides* species	ID	60, 100 (10)	100 (10)	ID	OB 69 (13)	OB 73 (15)	ID
Fusobacterium species[b]	OB 91 (11)	OB 90 (20)	OB 100 (16)	77, 92, 100 (13)	OB 94 (17)	OB 76 (17)	OB 90 (21)
Peptostreptococcus species	OB 87 (15)	OB 91 (11)	OB 100 (13)	OB 100 (13)	OB 78 (14)	OB 77 (13)	ID
Clostridium species	OB 92 (13)	73, 91, 100 (11)	ID	100 (12)	ID	92, 100 (12)	100 (10)
Gram-positive rods	OB 100 (12)	OB 100 (15)	ID	OB 90 (10)	OB 100 (14)	OB 92 (13)	OB 100 (15)

[a] Percent agreement within 1, 2, or 3 or more doubling dilutions [for those strains for which the MIC was not within the ranges tested, the percent agreement in off-boundary (OB) comparisons]. Values in parentheses are number of comparisons. ID, insufficient data (fewer than 10 comparisons).

[b] The percent agreement for *Fusobacterium* species is based on tail-beginning concentrations. This conforms to the NCCLS recommendation of determining MIC endpoints when there is a substantial reduction of growth, a barely visible haze, or both (9).

TABLE 6. Agreement between SG and AD MICs

Organism	% Agreement[a]						
	Chloramphenicol	Ciprofloxacin	Imipenem	Metronidazole	Win 57273	Clindamycin	Sparfloxacin
Bacteroides fragilis	100 (12)	90, 100 (10)	ID	75, 100 (12)	100 (11)	81, 100 (42)	96, 100 (49)
Other species of the *Bacteroides fragilis* group	100 (19)	88, 100 (17)	100 (11)	90, 100 (19)	94, 100 (17)	85, 94, 100 (47)	94, 97, 100 (70)
Pigmenting *Bacteroides* species	NT[b]	NT	NT	NT	NT	OB 100 (12)	100 (13)
Other *Bacteroides* species	NT	NT	NT	NT	NT	OB 93 (15)	94, 100 (16)
Fusobacterium species[c]	100 (10)	ID	ID	ID	ID	OB 100 (27)	97, 100 (32)
Clostridium species	100 (11)	ID	82, 100 (11)	ID	ID	81, 94, 100 (16)	88, 94, 100 (17)
Gram-positive rods	92, 100 (13)	ID	ID	ID	ID	OB 100 (15)	82, 100 (11)
Peptostreptococcus species	NT	NT	NT	NT	NT	80, 100 (10)	93, 93, 100 (14)

[a] Percent agreement within 1, 2, or 3 or more doubling dilutions [for those strains for which the MIC was not within the ranges tested, the percent agreement in off-boundary (OB) comparisons]. Values in parentheses are number of comparisons. ID, insufficient data (fewer than 10 comparisons).
[b] NT, not tested.
[c] The percent agreement for *Fusobacterium* species is based on tail-beginning concentrations. This conforms to the NCCLS recommendation of determining MIC endpoints when there is a substantial reduction of growth, a barely visible haze, or both (9).

misleading to discuss the error rates of the SGE technique. For example, a MIC of 31 μg/ml (which would be read as susceptible for a drug with a breakpoint of 32 μg/ml) by the SGE technique would be labeled a "very major error" if the MIC by the AD test was read as 64 μg/ml (i.e., resistant), even though the AD endpoint could be anywhere from 32 to 128 μg/ml. Particularly because our study indicated an "upward bias" in the determination of MICs by the AD technique (15), there would be many occasions in which a case such as that outlined above would be labeled a "very major error." Use of these interpretive criteria to compare these two methods would be misleading and would not contribute any useful information.

The SGE technique tested in this study retains the advantages of an AD system (in particular, good growth of fastidious organisms), incorporates some of the laborsaving advantages of the simpler techniques, and appears to be a useful alternative to the AD system for the organisms and antimicrobial agents investigated in these studies. Elimination of the need for serial dilution plates can result in substantial reductions in the time and materials needed to perform susceptibility tests. Also, reading of the MIC from a continuous gradient of drug concentrations has the potential for improved sensitivity and reproducibility.

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TABLE 7. Mean TECs and coefficients of variation for two control strains

Antimicrobial agent	*B. fragilis*[a]		*B. thetaiotaomicron*[a]	
	Mean TEC (μg/ml)	CV (%)	Mean TEC (μg/ml)	CV (%)
Cloramphenicol	6.4 (5.4–7)[b]	21	6.7 (5.7–7.3)	15
Ceftizoxime	105 (82–117)	22	48 (41–55)	20
Ciprofloxacin	3.1 (2.8–3.3)	15	14 (12–16)	18
Metronidazole	0.67 (0.6–0.78)	16	2.9 (2.6–3.4)	15

[a] Mean TEC and coefficient of variation (CV) calculated from all data.
[b] Values in parentheses are ranges of mean values obtained by three different technicians.

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