

Evaluation of Broth Microdilution Susceptibility Results for Anaerobic Organisms by Use of a Rapid Direct Colony Inoculum

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A direct colony inoculum suspension procedure was compared with the overnight suspension procedure recommended for the broth microdilution anaerobic commercial system (Micro-Media Systems, Inc., Potomac, Md.). Six National Committee for Clinical Laboratory Standards-recommended quality control organisms, *Bacteroides fragilis* ATCC 25285, *Clostridium perfringens* ATCC 13124, *Bacteroides thetaiotaomicron* ATCC 29741, *Bacteroides vulgatus* ATCC 29327, *Peptococcus magnus* ATCC 29328, *Peptococcus asaccharolyticus* ATCC 29743, and 50 anaerobic clinical isolates were tested against seven commonly tested antimicrobial agents. The minimum inhibitory concentration results from each suspension method (using the quality control organisms) were identical in 18 (78%) instances, and within $\pm 1 \log_2$ dilution in 96% of the comparisons. Results with the fresh clinical isolates also compared satisfactorily with the overnight procedure (97% were identical or within one dilution). The Wilkins-Chalgren test medium failed to support the growth of most anaerobic gram-positive cocci and *Bacteroides melaninogenicus* strains.

Susceptibility testing on anaerobic bacteria isolated from clinical specimens is of particular importance in the treatment of bacteremia, endocarditis, osteomyelitis, brain abscess, and serious infections of normally sterile body fluids or deep surgical wounds (5). In addition, susceptibility testing on anaerobes may be important since resistance to many empirically selected antimicrobial agents has been observed (13). Certainly, anaerobic susceptibility testing can be extremely useful for patient care, but it would be an even more useful procedure if the test results were more rapidly available. Bourgault et al. (4) have shown that early susceptibility results enable a physician to initiate appropriate therapy earlier in a patient's illness.

Methods used to obtain a direct rapid susceptibility report have been studied on aerobic clinical isolates (3) and blood culture isolates (6, 9, 10, 12). Other investigators (1, 2) have shortened the inoculum preparation time and have obtained a standardized suspension by using a commercially available inoculation wand. These cited studies have shown that reducing the incubation time of the inoculum yields results comparable to those with the overnight or standardized procedure and can provide earlier susceptibility information to a physician.

The purpose of this study was to determine whether MIC results on anaerobic microorganisms with a direct standardized colony suspension procedure are comparable to those obtained with the overnight standardized inoculum procedure.

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Three quality control strains, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, and three additional reference strains, *Bacteroides vulgatus* ATCC 29327, *Peptococcus magnus* ATCC 29328, and *Peptococcus asaccharolyticus*

ATCC 29743, were used to evaluate the direct colony suspension procedure (11). Subcultures of stocks were maintained in skim milk at -70°C . Each week, a vial of each culture was thawed and subcultured on pre-reduced anaerobically sterilized-supplemented brucella 5% defibrinated sheep blood agar plates (Anaerobe Systems, Santa Clara, Calif.) and incubated at 37°C for 48 h in a GasPak Jar (BBL Microbiology Systems, Cockeysville, Md.). In addition, the direct colony suspension procedure was tested with 50 fresh clinical isolates: 20 isolates of *B. fragilis* group species, 8 isolates of *Bacteroides* spp., 7 isolates of *Clostridium* spp., 5 isolates of *Fusobacterium* spp., 4 isolates of nonsporulating, gram-positive rods, 3 isolates of *Peptostreptococcus* spp., and 3 isolates of *Peptococcus* spp.

The antimicrobial concentration ranges (micrograms per milliliter) and antimicrobial agents used in the broth microdilution anaerobic MIC trays (Micro-Media Systems, Inc., Potomac, Md.) were: carbenicillin, 8.0 to 512; cefoxitin, 1.0 to 64; chloramphenicol, 0.5 to 32; clindamycin, 0.25 to 16; penicillin, 0.06 to 4; tetracycline, 0.25 to 16; and metronidazole, 0.25 to 16. The antimicrobial agents were diluted in Wilkins-Chalgren broth (7, 8).

Three to five well-isolated colonies were picked from a PRAS-supplemented brucella blood agar plate and inoculated into 5 ml of PRAS-thioglycolate 135C supplemented with hemin and vitamin K (Anaerobe Systems) and incubated overnight at 35°C . The overnight broth culture was diluted with sterile pre-reduced (boiled and cooled) isotonic saline with 0.02% Tween 80 to a turbidity equivalent to a no. 2 McFarland standard (6×10^8 CFU/ml). Then 0.6 ml of the standardized suspension was pipetted into 15 ml of isotonic saline with 0.02% Tween 80. When the inoculum was not sufficient to match a no. 2 McFarland standard, the suspension was diluted to a no. 1 McFarland standard and 1 ml was pipetted into isotonic saline with 0.02% Tween 80. The MIC trays were inoculated within 15 min after the inoculum was prepared. The direct colony suspension was prepared by picking a sufficient number of well-isolated colonies (four to five) from a 48-h PRAS-supplemented brucella blood agar plate and suspending them in 2 ml of PRAS-thioglycollate

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TABLE 1. Comparison of on-scale^a MIC results with six NCCLS quality control strains

Antibiotic	ATCC strain ^b (no. of tests)	Overnight suspension		Direct colony suspension
		MIC range (μg/ml)	MIC mode (μg/ml)	MIC mode (μg/ml)
Carbenicillin	<i>B. fragilis</i> (60)	≤8–32	16	16
	<i>B. thetaiotaomicron</i> (60)	≤8–64	32	16
Cefoxitin	<i>B. fragilis</i> (60)	2–8	4	4
	<i>B. thetaiotaomicron</i> (60)	4–32	16	16
	<i>B. vulgatus</i> (60)	2–4	2	2
Chloramphenicol	<i>B. fragilis</i> (60)	2–8	4	4
	<i>B. thetaiotaomicron</i> (60)	2–16	4	4
	<i>C. perfringens</i> (60)	2–4	4	4
	<i>B. vulgatus</i> (60)	1–4	2	2
	<i>P. magnus</i> (37)	1–4	2	2
	<i>P. asaccharolyticus</i> (40)	1–4	2	4
Clindamycin	<i>B. fragilis</i> (60)	0.5–4	2	2
	<i>B. thetaiotaomicron</i> (60)	1–4	2	2
Penicillin	<i>B. vulgatus</i> (60)	≤0.25–1	0.5	1
Tetracycline	<i>B. fragilis</i> (60)	0.5–2	1	0.5
	<i>B. thetaiotaomicron</i> (60)	4–16	8	2
	<i>B. vulgatus</i> (60)	2–8	4	4
	<i>P. magnus</i> (37)	≤0.25–1	0.5	0.5
Metronidazole	<i>B. fragilis</i> (60)	≤0.25–1	0.5	0.5
	<i>B. thetaiotaomicron</i> (60)	≤0.25–1	0.5	0.5
	<i>B. vulgatus</i> (60)	≤0.25–1	0.5	0.5
	<i>P. magnus</i> (37)	0.5–2	1	1
	<i>P. asaccharolyticus</i> (40)	1–4	2	2

^a On-scale results are only those results which fall within the scale of antibiotic values listed by the manufacturer.

^b Strains are identified by ATCC number in the text.

135C supplemented with hemin and vitamin K to match a no. 2 McFarland standard. Then 0.6 ml of inoculum was pipetted into 15 ml of isotonic saline with 0.02% Tween 80 as described above. The trays were also inoculated within 15 min after the inoculum was prepared.

According to the manufacturer's package insert, the MIC panels were prerduced in GasPak jars for a minimum of 4 h before inoculation. The inoculated trays were placed in 150-mm GasPak jars and incubated at 35°C for 48 h. The MIC for each antimicrobial agent was the lowest concentration inhibiting visible bacterial growth. For measuring the reproducibility of the test procedures, each National Committee for Clinical Laboratory Standards (NCCLS)-recommended organism was tested in triplicate on 20 different days, and each of the 50 clinical anaerobic isolates was tested on 20 different days.

With the use of six NCCLS-recommended quality control organisms, a total of 23 on-scale MIC comparisons were available for comparison between the direct colony suspension procedure and the overnight suspension method (Table 1). In 18 (78%) comparisons, mode MIC results from both procedures were identical. Four were within +1 log₂ dilution (two lower, two higher) of each other, thus 22 (96%) comparisons were considered acceptable.

Results with the 50 clinical anaerobic isolates are shown in Table 2. A total of 30 on-scale MIC comparisons could be made. In 23 (77%) comparisons, the results were identical. Six additional comparisons were within ±1 log₂ dilution (three lower, three higher) of the overnight suspension procedure (97% of results). Tetracycline showed the greatest

variation between methods, two of four (50%) with the NCCLS control organisms and three of six (50%) in the studies with the fresh clinical isolates. Major discrepancies (±2 log₂ dilutions) between the two methods were only observed with tetracycline.

The Wilkins-Chalgren medium used in this broth microdilution system did not adequately support the growth of anaerobic cocci. In many cases (40% of attempts), *P. magnus* and *P. asaccharolyticus* failed to grow (Tables 1 and 2). Data obtained with the clinical isolates showed that this broth microdilution system also failed to support the growth of *Bacteroides melaninogenicus* (7, 12).

An earlier study (7) showed that the commercially available Micro-Media Systems frozen microdilution anaerobe panels are comparable to the NCCLS reference method and are an acceptable alternative for obtaining MIC results. However, the method can take as long as 3 days, i.e., 1 day for preparation of the standard inoculum and 2 days for incubation of the trays. Patients with serious clinical infections could potentially benefit if MICs could be made available earlier to assist the physician in the selection of appropriate antimicrobial chemotherapy.

Investigators have reduced the incubation period of the inoculum of rapidly growing aerobic organisms to obtain an earlier susceptibility report (1, 2, 6, 9). Several investigators have shown that in seeking consistent results, the physiological state of the bacterial cells in the inoculum is not as important as the number of standardized viable cells or the density of the inoculum (1, 2). These studies indicate that the direct colony suspension procedure yields results that are

TABLE 2. Comparisons of on-scale^a MIC results with 50 clinical isolates

Antimicrobial agent	Test organism	No. tested	Overnight suspension		Direct colony suspension
			MIC range (μg/ml)	MIC mode (μg/ml)	MIC mode (μg/ml)
Carbenicillin	<i>B. fragilis</i> group spp.	40	≤8-64	16	16
	<i>Bacteroides</i> spp.	16	≤8-32	16	16
Cefoxitin	<i>B. fragilis</i> group spp.	40	4-32	16	16
	<i>Bacteroides</i> spp.	16	2-16	8	16
	<i>Clostridium</i> spp.	14	2-16	8	8
	<i>Fusobacterium</i> spp.	10	8-64	32	32
Chloramphenicol	<i>B. fragilis</i> group spp.	40	2-8	4	4
	<i>Bacteroides</i> spp.	16	0.5-2	1	1
	<i>Clostridium</i> spp.	14	1-4	2	4
	<i>Fusobacterium</i> spp.	10	2-8	4	4
	Nonsporulating gram-positive rods	8	0.5-2	1	1
	<i>Peptococcus</i> spp.	4	1-4	2	2
Clindamycin	<i>B. fragilis</i> group spp.	40	0.5-4	2	2
	<i>Bacteroides</i> spp.	16	≤0.25-1	0.5	0.5
	<i>Clostridium</i> spp.	14	0.5-4	2	2
	<i>Fusobacterium</i> spp.	10	1-8	4	2
Penicillin	<i>Bacteroides</i> spp.	16	0.5-2	1	1
	<i>Fusobacterium</i> spp.	10	≤0.06-0.25	0.12	0.12
Tetracycline	<i>B. fragilis</i> group spp.	40	2-16	8	4
	<i>Bacteroides</i> spp.	16	0.5-8	4	4
	<i>Clostridium</i> spp.	14	2-16	8	16
	<i>Fusobacterium</i> spp.	10	2-16	8	8
	Nonsporulating gram-positive rods	8	0.5-4	2	8
	<i>Peptococcus</i> spp.	4	≤0.25-2	1	1
Metronidazole	<i>B. fragilis</i> group spp.	40	≤0.25-2	2	2
	<i>Bacteroides</i> spp.	16	≤0.25-2	1	1
	<i>Clostridium</i> spp.	14	1-4	2	1
	<i>Fusobacterium</i> spp.	10	≤0.25-4	1	1
	Nonsporulating gram-positive rods	8	2-8	4	4
	<i>Peptococcus</i> spp.	4	1-4	2	2

^a On-scale results are only those results which fall within the scale of antibiotic values listed by the manufacturer.

comparable to those with the overnight suspension procedure. In the present study, a direct colony suspension of anaerobic organisms was used to significantly decrease the inoculum preparation time and produce an earlier antimicrobial susceptibility result.

Tetracycline, an antimicrobial agent of little therapeutic use against anaerobic infections, showed the greatest tendency for discrepancies between the direct colony and overnight procedures. In previous studies (7, 8, 12), tetracycline also gave inconsistent results, supporting the possible deletion of this drug from routine testing.

In conclusion, the direct colony suspension procedure, with a commercial anaerobe MIC test panel, yielded results comparable to those with the overnight suspension procedure; this makes it possible to set up MIC tests immediately, rather than waiting for the overnight broth growth suspension.

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