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Quality Control Guidelines for Testing Cefotetan in the Reference Agar Dilution Procedure for Susceptibility Testing of Anaerobic Bacteria

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Reference values for quality control of in vitro susceptibility tests with cefotetan against anaerobic bacteria were determined in two independent multilaboratory studies with the approved National Committee for Clinical Laboratory Standards agar dilution method and three control strains (*Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124). The results of the two studies were in agreement. The recommended MIC control limits for *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 are 4.0 to 16 μg/ml and 32 to 128 μg/ml, respectively. MICs for *C. perfringens* ATCC 13124 were too variable to be useful for controlling tests with cefotetan.

Cefotetan is a long-acting cephamycin recommended for use in mixed aerobic and anaerobic intra-abdominal and pelvic infections (1, 6). Cefotetan reference values for quality control of in vitro susceptibility tests against bacteria that grow aerobically have been established (2).

To provide clinical microbiologists with cefotetan MIC quality control guidelines for use in anaerobic susceptibility testing, two independent, multilaboratory studies were conducted. The first study (study A) satisfied the requirements outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (4). The following institutions participated in study A: St. Vincent Medical Center, Portland, Oreg.; Centers for Disease Control, Atlanta, Ga.; University of Indiana Medical Center, Indianapolis; St. Francis Medical Center, Wichita, Kans.; and The Clinical Microbiology Institute, Tualatin, Oreg. The second study (study B) involved replicate testing of the control organisms on plates which were freshly prepared and tested for 20 consecutive days. These tests were performed at Sinai Samaritan Medical Center (Mt. Sinai Campus), Milwaukee, Wis., and at ICI Pharmaceuticals Group, Division of ICI Americas Inc., Wilmington, Del. In both studies the reference agar dilution was followed as prescribed elsewhere (3).

All tests in both studies were performed by the anaerobe agar dilution reference procedure for susceptibility testing of anaerobic bacteria as described by the NCCLS (3). Replicate tests were performed with *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124. For each control strain, 20 cefotetan MICs were generated by each laboratory participating in study A. Study B participants determined 156, 90, and 92 cefotetan MICs with the *B. fragilis*, *B. thetaiotaomicron*, and *C. perfringens* quality control strains, respectively.

All participating laboratories used the lot of Wilkins-Chalgren agar that was available in the laboratory at the time of the study. Cefoxitin was tested by all laboratories to provide a procedural control. The two *Bacteroides* species provided cefoxitin endpoints that were within the control limits defined by the NCCLS (3). However, two laboratories (study A) reported cefoxitin MICs for the *C. perfringens* isolate that were 1 dilution greater than the accepted upper limit of 1.0 μg/ml.

Table 1 presents the cefotetan MIC endpoint determination for *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741. For the *B. fragilis* control strain, all 256 MIC determinations fell within a range of 4.0 to 16 μg/ml, i.e., 1 doubling dilution above or below the 8.0-μg/ml modal MIC. A quality control range of 4.0 to 16 μg/ml is recommended for *B. fragilis* ATCC 25285, and those limits have been recommended in the most recent NCCLS informational supplement (5). When *B. thetaiotaomicron* ATCC 29741 was tested in study A, the mode was not readily identified, since the cefotetan MICs were equally distributed at 64 and 128 μg/ml. In the second study, 89 of 90 (99%) MICs for the *B. thetaiotaomicron* strain were 64 μg/ml, and that defined the mode. Since 189 of 190 MICs were either 64 or 128 μg/ml, the control range could be defined to include only 2 doubling dilutions (64 and 128 μg/ml). More traditional control limits would include 1 doubling dilution on each side of the modal value. We recommend cefotetan MIC control limits of 32 to 128 μg/ml for tests with *B. thetaiotaomicron* ATCC 29741. Those control limits have been recommended in the recent NCCLS informational supplement (5).

More variability was seen when the results for *C. perfringens* ATCC 13124 were examined. Cefotetan MICs ranged from the lowest concentration tested (≤0.03 or ≤0.015 μg/ml) to 2.0 μg/ml (Table 2). Because of the wide distribution of MICs, modal MICs are not listed. Because the *C. perfringens* quality control strain does not appear useful for monitoring the performance of cefotetan susceptibility tests, no control limits are recommended. Sutter et al. also observed

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TABLE 1. Cefotetan agar dilution MICs for two *Bacteroides* control strains[a]

Organism tested	Study[b]	Total no. of determinations	MIC (μg/ml)	No. of determinations (% of total)	Mode MIC (μg/ml)
B. fragilis ATCC 25285	A	100	4.0	20 (20)	8.0
			8.0	63 (63)	
			16.0	17 (17)	
	B	156	4.0	36 (23)	8.0
			8.0	120 (77)	
	Combined	256	4.0	56 (22)	8.0
			8.0	183 (71)	
			16.0	17 (7)	
B. thetaiotaomicron ATCC 29741	A	100	64	50 (50)	64
			128	50 (50)	
	B	90	32	1 (1)	64
			64	89 (99)	
	Combined	190	32	1 (<1)	64
			64	139 (73)	
			128	50 (26)	

[a] Determined in two independent multicenter studies by using Wilkins-Chalgren agar without added blood or other supplements.
[b] Study A, A five-laboratory coordinated study which fulfills the requirements of the NCCLS (4); study B, an extended study contributing additional MICs from two other facilities.

inconsistencies in results when this reference organism was tested against other β-lactam antibiotics (8).

Study B participants also performed tests with Wilkins-Chalgren agar containing 5% sheep blood. With the added blood, cefotetan MICs for the two *Bacteroides* species fell within the MIC control ranges that are recommended herein (data not shown). Even with added blood, results for *C. perfringens* ATCC 13124 were too erratic to provide useful control information. If the addition of sheep blood is required for growth of the organism being tested, the activity of cefotetan will probably not be affected.

It is possible that the inconsistency of results observed with *C. perfringens* ATCC 13124 is related to difficulties in obtaining a standardized inoculum due to the cellular morphology or growth characteristics of the organism (7) or to difficult-to-interpret endpoints (8) or both. These possibilities are currently under investigation in our laboratories. Alternative control strains should be considered in the near future.

TABLE 2. Cefotetan agar dilution MICs for *Clostridium perfringens* ATCC 13124[a]

Study[b]	Total no. of determinations	MIC (μg/ml)	No. of determinations (% of total)
A	100	≤0.03	24 (24)
		0.06	15 (15)
		0.12	17 (17)
		0.25	4 (4)
		0.5	3 (3)
		1.0	16 (16)
		2.0	21 (21)
B	92	≤0.015	1 (1)
		0.03	14 (15)
		0.06	5 (5)
		0.25	2 (2)
		0.5	38 (42)
		1.0	29 (32)
		2.0	3 (3)
Combined	192	≤0.03	39 (20)
		0.06	20 (10)
		0.12	17 (9)
		0.25	6 (3)
		0.5	41 (21)
		1.0	45 (23)
		2.0	24 (13)

[a] Determined in two independent multicenter studies by using Wilkins-Chalgren agar without added blood or other supplements.
[b] Study A, A five-laboratory coordinated study which fulfills the requirements of the NCCLS (4); study B, an extended study combining 92 MICs from two other facilities.

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