

## Quality Control Guidelines for Cefdinir, Cefepime, Cefetamet, Cefmetazole, Cefpodoxime, Cefprozil, and Clinafloxacin (CI-960) for Various National Committee for Clinical Laboratory Standards Susceptibility Testing Methods

MARTHA J. BALE, RONALD N. JONES,\* AND THE QUALITY CONTROL STUDY GROUP†

University of Iowa College of Medicine, Iowa City, Iowa 52242

Received 8 April 1993/Returned for modification 11 May 1993/Accepted 14 June 1993

Several multilaboratory studies to determine quality control (QC) ranges for a variety of National Committee for Clinical Laboratory Standards (NCCLS) susceptibility tests are summarized. Replicate testing used multiple lots of media and antimicrobial disks in accordance with NCCLS recommendations, including the appropriate medium modifications for tests with *Haemophilus* spp. and *Neisseria gonorrhoeae*. QC ranges for MIC and disk diffusion testing of *N. gonorrhoeae* ATCC 49226 were proposed for cefepime, cefetamet, cefmetazole, and cefpodoxime. Disk diffusion QC ranges for *Haemophilus influenzae* ATCC 49247 or ATCC 49766 were recommended with cefepime, cefetamet (10- and 30- $\mu$ g disks), cefmetazole, cefpodoxime, and cefprozil. Disk diffusion QC ranges for *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 with cefdinir and clinafloxacin and those for *Pseudomonas aeruginosa* ATCC 27853 with clinafloxacin were also proposed.

Modifications of standard methods have been made by the National Committee for Clinical Laboratory Standards (NCCLS) for susceptibility testing of *Haemophilus influenzae* and *Neisseria gonorrhoeae* (9, 10). Both of these organisms require a medium other than Mueller-Hinton (MH), i.e., Haemophilus Test Medium (HTM) or GC agar with a 1% defined supplement. A recent survey by the College of American Pathologists has shown wide adoption of HTM for clinical antimicrobial susceptibility testing of *Haemophilus* spp. (6). As modifications to the standard procedures and new drugs become available with interpretive criteria (3), quality control (QC) ranges need to be established. These QC ranges help to ensure reliable and reproducible in vitro test results.

Several of the newer antimicrobial agents reported here have had broth dilution MIC QC ranges established for *H. influenzae* ATCC 49247 (cefetamet, cefpodoxime, and clinafloxacin) (8) and disk diffusion QC ranges established for cefdinir and clinafloxacin (2). This study expands the information by proposing QC ranges for cefepime, cefetamet, cefmetazole, and cefpodoxime disk diffusion tests with *H. influenzae* ATCC 49247 on HTM and for disk diffusion and MIC testing of *N. gonorrhoeae* ATCC 49226 on GC medium. In addition, recommendations for disk diffusion QC ranges for the routine nonfastidious QC strains for cefdinir and clinafloxacin on MH agar are established. Cefprozil QC ranges for *H. influenzae* ATCC 49766 are proposed for disk diffusion and MIC testing with HTM.

The study design followed the recommendations of NC-

CLS document M23-T (11) for determining QC parameters. Six separate laboratories participated in these evaluations. Disk diffusion susceptibility tests were performed according to the standards recorded in NCCLS M2-A4 (9). QC strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were tested in or on MH media. *H. influenzae* ATCC 49247 was tested on HTM with incubation in 5 to 7% CO<sub>2</sub> for 16 to 18 h. *N. gonorrhoeae* ATCC 49226 was tested on GC agar in 5 to 7% CO<sub>2</sub> and incubated for 20 to 24 h. Broth microdilution susceptibility tests were performed according to NCCLS standards (10). HTM was used for testing *Haemophilus* spp., and the GC agar medium was used for testing *N. gonorrhoeae*. *H. influenzae* ATCC 49766 was utilized as the control strain when cefprozil was tested by both methods (9, 10).

Six lots of MH agar were obtained from three manufacturers (BDMS, Cockeysville, Md.; Difco, Detroit, Mich.; and Oxoid, USA, Inc., Columbia, Md.), six lots of GC medium were obtained from two manufacturers (BDMS and Difco), and seven lots of HTM were obtained from four manufacturers (Accumedia, Cockeysville, Md.; BDMS; Difco; and Oxoid). Each of the six participant laboratories performed 20 replicate tests with a unique lot of medium and 5 replicate tests with a lot of medium common to all laboratories. For disk diffusion testing, three lots of disks from at least two manufacturers (BDMS and Difco) were used for each drug (two lots only were used with cefprozil because of an interim change in the NCCLS M23 standard). The established QC ranges for ampicillin on HTM and tetracycline on GC agar were used to control each lot and experiment. Results with the common lot from each laboratory were compared for significant variation. The statistical methods of Barry et al. and Gavan et al. were used to set the proposed MIC QC ranges (4, 7). The statistic for determining zone diameter ranges is the overall median zone size  $\pm$  one-half of the median of the individual laboratory ranges. An interval containing 95% of test values, as currently

\* Corresponding author.

† Members of the Quality Control Study Group are P. R. Murray, Barnes Hospital, Washington University, St. Louis, Mo.; J. A. Washington, The Cleveland Clinic Foundation, Cleveland, Ohio; E. H. Gerlach, St. Francis Regional Medical Center, Wichita, Kans.; C. Thornsberry, Institutes of Microbiology Research, Franklin, Tenn.; J. Hindler, UCLA Medical Center, Los Angeles, Calif.; and G. V. Doern, University of Massachusetts, Worcester, Mass.

TABLE 1. MIC QC results from five or six laboratories using unique and one common lot of media<sup>a</sup>

QC organism	Antimicrobial agent	No. of occurrences <sup>b</sup> at MIC ( $\mu\text{g/ml}$ ) of:								
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
<i>N. gonorrhoeae</i> <sup>c</sup>	Cefepime	[13	113	24]						
	Cefetamet	[25	50	50	25] <sup>d</sup>					
	Cefmetazole						[20	71	59]	
	Cefpodoxime	10	[28	107	5]					
<i>H. influenzae</i> <sup>e</sup>	Cefprozil						[40	110	0]	

<sup>a</sup> Results for two organisms are presented. All results are listed because of no significant variation between unique and common-lot results.

<sup>b</sup> Brackets indicate the proposed QC range.

<sup>c</sup> All tests were on GC agar with the ATCC 49226 strain.

<sup>d</sup> A bimodal MIC distribution required a 4-log<sub>2</sub> dilution range.

<sup>e</sup> All tests were in HTM broth with the ATCC 49766 strain.

preferred by the NCCLS, was used for selecting disk diffusion QC zone ranges.

The zone diameters of the different lots of antimicrobial disks did not vary significantly for any of the drugs tested. The control drug QC results for all lots of media used were within the established NCCLS limits (9, 10). All ampicillin disk zones for tests on HTM were within the range of 13 to 21 mm. Tetracycline zone diameters on GC agar were within the established range for 149 of 150 or 99.3% of the results. In several cases, an individual laboratory's results significantly varied from the all-laboratory median value. In these cases, that laboratory's data were not used for QC range selection. The remaining number of replicate tests (usually 375 zone diameters) was sufficient for determining the QC ranges (10).

Table 1 summarizes the MIC testing of *N. gonorrhoeae* ATCC 49226 on GC agar with 1% defined supplement. The recommended QC range was the modal MIC  $\pm 1$  log<sub>2</sub> dilution for all drugs except cefetamet. For this drug, the mode was between two adjacent dilution steps, resulting in a

4-dilution QC range, e.g., 0.015 to 0.12  $\mu\text{g/ml}$ . For all antimicrobial agents tested, the proposed range included 93.3 (cefpodoxime) to 100% of the MICs. The results for cefprozil with *H. influenzae* ATCC 49766 demonstrated minimal medium lot variation by MIC testing.

Table 2 summarizes the disk diffusion testing of *N. gonorrhoeae* ATCC 49226 on six lots of GC agar. One laboratory had a significant variation from the other laboratories' results on the common lot of medium with cefetamet (30  $\mu\text{g}$ ). Only 375 replicate tests were used for this analysis, rather than the 450 replicates for all other drugs. There was minimal intralaboratory variation for all disks except cefetamet (30- $\mu\text{g}$ ) disks, as demonstrated by the one-half median range statistic of 2 mm (data not shown). However, modest lot-to-lot variation did occur and required widened QC ranges (6 to 10 mm) to include 95% of all results.

Results of disk diffusion tests for *H. influenzae* ATCC 49247 are also shown in Table 2. One laboratory had a significant variation of results with cefetamet disks, and its data were eliminated from the analysis. Within media, lot

TABLE 2. Replicate disk diffusion susceptibility results for *N. gonorrhoeae* ATCC 49226, *H. influenzae* ATCC 49247, and three other QC strains

QC organism	Antimicrobial agent (disk content [ $\mu\text{g}$ ])	No. of tests	Median zone (mm)	95% CI <sup>a</sup> (mm)	% Results in CI range
<i>N. gonorrhoeae</i>	Cefepime (30)	450	40	37-46	95.6
	Cefetamet (10)	450	38	34-44	97.8
	Cefetamet (30)	375 <sup>b</sup>	45	41-48	95.2
	Cefmetazole (30)	450	34	31-36	95.4
	Cefpodoxime (10)	450	39	36-42	95.3
<i>H. influenzae</i>	Cefepime (30) <sup>c</sup>	450	29	26-31	97.1
	Cefetamet (10) <sup>c</sup>	375 <sup>b</sup>	26	23-28	95.4
	Cefetamet (30) <sup>c</sup>	375 <sup>b</sup>	27	24-30	96.7
	Cefmetazole (30) <sup>c</sup>	450	18	17-20	95.1
	Cefpodoxime (10) <sup>c</sup>	450	29	25-31	98.7
	Cefprozil (30) <sup>d</sup>	300	23	20-27	96.7
<i>E. coli</i>	Cefdinir (5)	450	26	24-28	98.9
	Clinafloxacin (5)	375 <sup>b</sup>	38	35-40	95.7
<i>P. aeruginosa</i>	Clinafloxacin (5)	375 <sup>b</sup>	33	30-36	96.5
<i>S. aureus</i>	Cefdinir (5)	375 <sup>b</sup>	34	28-37	97.6
	Clinafloxacin (5)	375 <sup>b</sup>	35	32-39	96.0

<sup>a</sup> CI, confidence interval.

<sup>b</sup> One laboratory's results differed significantly from all others and were omitted.

<sup>c</sup> With QC strain ATCC 49247.

<sup>d</sup> With QC strain ATCC 49766 and new NCCLS M23 guidelines for two disk lots and increased common-lot testing.

variation was larger for cefetamet (30 µg) than for other drugs. Lot-to-lot variation did require a slight expansion of the QC ranges to achieve an interval including 95% of values; the expansion was from 4 or 6 to 6 or 7 mm. The disk diffusion zone diameter range for cefprozil with *H. influenzae* ATCC 49766 indicated by the median's statistical calculation (1, 2, 5, 7, 9) was expanded (20 to 27 mm) in order to encompass ≥95% of the test results (Table 2).

Cefdinir and clinafloxacin (formerly CI-960) were tested against the susceptibility test QC organisms used for routine clinical procedures: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 (Table 2). Cefdinir is not active against *P. aeruginosa*. Within-lot variation of MH agar was minimal, e.g., the one-half median range statistics were ≤2 mm (data not shown). However, lot-to-lot variation was extreme with *S. aureus* with cefdinir disks. The median ± one-half the median range statistic, previously used by the NCCLS for QC range selection, only included 39.7% of the test results. The proposed QC range was increased to include 95% of the test results (28 to 37 mm).

In conclusion, our proposed MIC QC ranges for *N. gonorrhoeae* ATCC 49226 are as follows: cefepime, 0.015 to 0.06 µg/ml; cefetamet, 0.015 to 0.12 µg/ml; cefmetazole, 0.5 to 2 µg/ml; and cefpodoxime, 0.03 to 0.12 µg/ml. The proposed disk zone diameter QC ranges for *N. gonorrhoeae* ATCC 49226 are as follows: cefepime, 37 to 46 mm; cefetamet at 10 µg, 34 to 44 mm; cefetamet at 30 µg, 41 to 48 mm; cefmetazole, 31 to 36 mm; and cefpodoxime, 36 to 42 mm. The proposed QC ranges for disk diffusion susceptibility of *H. influenzae* ATCC 49247 are as follows: cefepime, 26 to 31 mm; cefetamet at 10 µg, 23 to 28 mm; cefetamet at 30 µg, 24 to 30 mm; cefmetazole, 17 to 20 mm; and cefpodoxime, 25 to 31 mm. The cefdinir 5-µg-disk range for *E. coli* ATCC 25922 should be 24 to 28 mm, and that for *S. aureus* ATCC 25923 should be 28 to 37 mm. The QC ranges for clinafloxacin 5-µg disks are 35 to 40 mm for *E. coli* ATCC 25922, 30 to 36 mm for *P. aeruginosa* ATCC 27853, and 32 to 39 mm for *S. aureus* ATCC 25923. The proposed QC ranges for cefprozil (*H. influenzae* ATCC 49766) are 1 to 4 µg/ml for the broth microdilution MIC test and 20 to 27 mm for the disk diffusion method.

We propose QC ranges for seven investigational antibiotics when tested by various NCCLS methods (9, 10). These results should expand the number of compounds which can be reliably tested with HTM and GC agar and by routine standard methods with MH agar (1, 2, 5, 9, 10). The ranges proposed should be considered tentative until adopted by national standards groups and subsequently published for at least 1 year (9, 10).

Our thanks to M. E. Erwin for the technical organization of the individual trials and P. Carney for word-processing assistance.

## REFERENCES

- Bale, M. J., R. N. Jones, M. E. Erwin, F. P. Koontz, E. H. Gerlach, P. R. Murray, and J. A. Washington. 1992. MIC quality control guidelines for *Haemophilus* susceptibility tests using cefdinir (FK482), cefepime, cefetamet, cefpirome, cefibuten, fleroxacin, temafloxacin, clarithromycin, RP59500, and trospectomycin. *J. Clin. Microbiol.* **30**:225-226.
- Bale, M. J., R. N. Jones, M. E. Erwin, F. P. Koontz, E. H. Gerlach, P. R. Murray, and J. A. Washington. 1992. Disk diffusion quality control guidelines for *Haemophilus* susceptibility tests using cefdinir, CI-960, fleroxacin, temafloxacin, and trospectomycin. *J. Clin. Microbiol.* **30**:744-755.
- Barrett, M. S., and R. N. Jones. 1992. Interpretive criteria for CI-960, fleroxacin and temafloxacin susceptibility tests with *Haemophilus influenzae*. *Eur. J. Clin. Microbiol. Infect. Dis.* **11**:463-465.
- Barry, A. L., P. C. Fuchs, R. N. Jones, and the Collaborative Antimicrobial Susceptibility Testing Group. 1989. Statistical criteria for selecting quality control limits for broth microdilution susceptibility tests with 39 different antimicrobial agents. *Diagn. Microbiol. Infect. Dis.* **12**:413-420.
- Doern, G. V., E. H. Gerlach, J. H. Jorgensen, P. R. Murray, C. Thornsberry, and J. A. Washington II. 1991. Quality control limits for disk diffusion and broth microdilution susceptibility tests with *Haemophilus* Test Medium. *Diagn. Microbiol. Infect. Dis.* **14**:485-494.
- Doern, G. V., and R. N. Jones. In vitro susceptibility test practices with *Haemophilus influenzae* among College of American Pathologists survey participants in the United States. *Diagn. Microbiol. Infect. Dis.* **17**:61-65.
- Gavan, T. L., R. N. Jones, A. L. Barry, P. C. Fuchs, E. H. Gerlach, J. M. Matsen, L. B. Reller, C. Thornsberry, and L. D. Thrupp. 1981. Quality control limits for ampicillin, carbenicillin, mezlocillin, and piperacillin disk diffusion susceptibility tests: a collaborative study. *J. Clin. Microbiol.* **14**:67-72.
- Jones, R. N., J. A. Washington, M. A. Pfaller, F. P. Koontz, E. H. Gerlach, and M. E. Erwin. 1991. Quality control guidelines for *Haemophilus* test medium MIC susceptibility tests with cefmetazole, cefpodoxime, CI-960 (PD127391, AM-1090), and RU29246. *J. Antimicrob. Chemother.* **27**:390-392.
- National Committee for Clinical Laboratory Standards. 1990. Performance standards for antimicrobial disk susceptibility test, 4th ed. Approved standard M2-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- National Committee for Clinical Laboratory Standards. 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- National Committee for Clinical Laboratory Standards. 1992. Development of in vitro susceptibility testing criteria and quality control parameters, 2nd ed. Tentative guideline M23-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.