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Quality Control Guidelines for BAL9141 (Ro 63-9141), an Investigational Cephalosporin, When Reference MIC and Standardized Disk Diffusion Susceptibility Test Methods Are Used

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BAL9141 is a novel cephalosporin with a broad spectrum of activity, including activity against methicillin-resistant staphylococci. This multicenter study was performed to establish quality control (QC) guidelines for susceptibility testing of BAL9141 in phase 3 clinical trials and after U.S. Food and Drug Administration approval. The proposed 3 or 4 $\log_2$ dilution MIC ranges encompass 97.8 to 100.0% of reported results, while the proposed 7- to 9-mm-zone-diameter QC ranges included 95.2 to 99.4% of the participant-reported disk diffusion results.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are usually resistant to other classes of antimicrobials, namely, aminoglycosides, fluoroquinolones, macrolides, and tetracyclines (6, 8). β-Lactams also pose a unique therapeutic problem due to the ability of MRSA to produce penicillinase and a low-affinity target penicillin-binding protein, PBP 2a (6, 8). Thus, the therapeutic options for treating MRSA infections have been limited to glycopeptides, quinupristin-dalfopristin, or linezolid (1, 6, 8). The treatment of MRSA infections became even more complex due to the emergence of strains with decreased susceptibility to glycopeptides, thus increasing the need to develop novel antimicrobials to treat MRSA infections (4, 8).

BAL9141 (formerly Ro 63-9141) is a pyrrolidinone-3-ylidenemethyl cephalosporin which has a broad spectrum of antimicrobial activity; most notable is the potent activity against methicillin-resistant staphylococci (2, 5, 7). BAL9141 activity against MRSA is due to the inhibition of PBP 2a and stability with respect to β-lactamase hydrolysis. BAL9141 has demonstrated excellent activity against MRSA, with MICs at which 50% and 90% of the isolates tested are inhibited in the range of 2 to 4 μg/ml (5, 7). BAL9141 also has notable activity against *Streptococcus* spp., *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria* spp., enterobacteriaceae, nonfermentative gram-negative bacilli, and anaerobes (2, 5, 7). This broad spectrum of activity plus the potency against MRSA makes BAL9141 a promising antimicrobial agent that has been advanced into human clinical trials. To determine the accurate assessment of the susceptibility test patterns for clinical isolates, quality control (QC) guidelines for BAL9141 will be required (9–12).

A multicenter study group was recruited for the development of MIC and disk diffusion QC guidelines for BAL9141. The QC study group consisted of laboratories at the University of Washington, Seattle; Denver Health Medical Center, Denver, Colo.; Strong Memorial Hospital, Rochester, N.Y.; University of Texas, Houston; The Cleveland Clinic Foundation, Cleveland, Ohio; University of Alberta, Edmonton, Alberta, Canada; TREK Diagnostics, Cleveland, Ohio; and JMI Laboratories, North Liberty, Iowa. Each laboratory followed the protocol based on National Committee for Clinical Laboratory Standards (NCCLS) M23-A2 guidelines (9) as well as the M7-A6 test method (10) for broth microdilution antimicrobial testing and the M2-A8 method (10) for antimicrobial disk diffusion testing.

The MIC portion of the study utilized frozen-form, reference broth microdilution panels prepared by TREK Diagnostics (Cleveland, Ohio). The panels contained four lots of cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich. [two lots]; Oxoid, Hampshire, United Kingdom; BBL, Sparks, Md.), three lots of cation-adjusted Mueller-Hinton broth (Difco, Oxoid, BBL) supplemented with 5% lysed horse blood, or four lots of *Haemophilus* test medium (Difco [two lots], Oxoid, BBL). The antimicrobial agents in this study were obtained as follows: BAL9141 was supplied by Basilea Pharmaceutica (Basel, Switzerland [study sponsor]), cefepime was from Bristol-Myers Squibb (Plainsboro, N.J.), and cefuroxime and vancomycin were from Sigma Chemical (St. Louis, Mo.) (the latter three compounds served as internal control agents). Each laboratory tested seven QC strains: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247 and 49766. All strains were tested daily for 10 days, generating 320 MICs (240 MIC results for *S. pneumoniae* ATCC 49619) per

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TABLE 1. Inter- and intralaboratory comparisons of BAL9141 MIC results for *S. aureus* ATCC 29213 in an eight-medical-center protocol[a]

MIC (μg/ml)	No. of occurrences for indicated laboratory code								Total
	A	B	C	D	E	F	G	H	
0.12									0
0.25	10	18	2	39	19	17	11	1	117[b]
0.5	30	22	36	1	21	23	29	39	201[b]
1			2						2[b]
2									0

[a] Protocol designed using NCCLS guidelines (9).
[b] Proposed 3 $\log_2$ dilution QC range (includes 100.0% of results).

TABLE 2. Inter- and intralaboratory comparisons of the BAL9141 (30-μg disk) zone diameter results for *S. pneumoniae* ATCC 49619 in an eight-medical-center protocol[a]

Zone diam (mm)	No. of occurrences for indicated laboratory code[b]								Total
	A	B	C	D	E	F	G	H	
30									0
31			2						2
32			19			2			21[c]
33	2		38			7	1	4	52[c]
34	6	4	1	9	11	8	2	8	49[c]
35	14	10		18	26	6	15	23	112[c]
36	20	22		22	11	5	18	12	110[c]
37	15	17		10	6	13	17	7	85[c]
38	3	7		1	6	9	6	3	35[c]
39						8	1	1	10[c]
40						2			2
41									0
Total	60	60	60	60	60	60	60	58	478

[a] Protocol designed using NCCLS guidelines (9).
[b] Median and range of zone diameter results (mm) for laboratory code A, 36 and 6, respectively; for B, 36 and 5; for C, 33 and 4; for D, 36 and 5; for E, 35 and 5; for F, 37 and 9; for G, 36 and 7; for H, 35 and 7; for total results, 36 and 10.
[c] Proposed QC zone diameter range (includes 99.2% of results).

QC organism tested. Multiple colony counts were also performed by subculturing in a quantitative manner onto drug-free plates. The counts ranged from 7.0×10^4 to 1.5×10^6 CFU/ml, with an average of 4.1×10^5 CFU/ml for all participating laboratories (target inoculum at 5.0×10^5 CFU/ml).

The disk diffusion portion of the study utilized three different lots of commercially prepared Mueller-Hinton agar and *Haemophilus* test medium (Remel, Lenexa, Kans.; BBL; Acumedia, Baltimore, Md.) and three different lots of Mueller-Hinton agar with 5% sheep blood (Remel, BBL [2 lots]). Two different disk lots of BAL9141 disks were prepared by the MAST Group (Merseyside, United Kingdom), and one lot of each of the commercially available cefuroxime and cefepime (BBL) and levofloxacin and vancomycin (Remel) disks was used for the internal control agents. Each laboratory tested six QC strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247 and 49766. All strains were tested daily for 10 days, generating two zones on each of three different medium lots for a total of ≥478 (≥420 minimum per NCCLS guidelines) zone diameter values per QC organism.

Proposed QC ranges were optimized to encompass ≥95% of all reported results, as recommended by NCCLS M23-A2 guidelines (9). The MIC and disk diffusion zone diameter results were tabulated and compared by intra- and interlaboratory analysis to determine potentially unacceptable technical variations. Broth or agar medium and disk lots were also compared to determine variations among manufacturers. No significant variations among laboratories, media, or disk lots were observed. All concurrent control drug results were within NCCLS QC ranges (12).

Table 1 shows an example of BAL9141 MIC distributions among the eight participant laboratories testing *S. aureus* ATCC 29213. The results for seven of the eight laboratories had a modal value of 0.5 μg/ml (62.8% of the total results), with MICs for each laboratory ranging from 2 to 3 $\log_2$ dilutions. The proposed MIC range was 0.25 to 1 μg/ml, which would encompass 100.0% of reported results. Similar results were obtained for *E. faecalis* ATCC 29212, with 46.2% of the total results at the modal value of 0.12 μg/ml. The MIC ranges for each laboratory were 1 to 4 $\log_2$ dilutions, and the proposed range was 0.06 to 0.5 μg/ml, which would encompass all reported results. The study results for *E. coli* ATCC 25922 showed the modal MIC to be 0.06 μg/ml (84.7% of the total results) and a proposed range of 0.03 to 0.12 μg/ml, which included 100.0% of the reported MIC results. *P. aeruginosa* ATCC 27853 had 63.1% of the reported results at the modal value of 2 μg/ml, with 97.8% of reported results in the proposed range of 1 to 4 μg/ml. The combined participant results for *S. pneumoniae* ATCC 49619 showed a modal value of 0.008 μg/ml (49.2% of the total results), but five of the eight laboratories reported modal values of 0.015 μg/ml (43.8% of the total results). With the bimodal MICs, the proposed 4 $\log_2$ dilution range of 0.004 to 0.03 μg/ml included all MIC results. *H. influenzae* ATCC 49247 had 49.4% of the reported results at 0.25 μg/ml, again with all reported results in the proposed range (0.12 to 1 μg/ml). The results for *H. influenzae* ATCC 49766 showed the modal value to be 0.03 μg/ml (75.0% of the total results), and the MIC ranges were 1 to 3 $\log_2$ dilution steps (proposed range, 0.015 to 0.06 μg/ml).

Table 2 shows an example of BAL9141 disk diffusion zone diameter distributions among the eight laboratories testing *S. pneumoniae* ATCC 49619. With 23.0% of the total results at the overall median value (36 mm), the proposed QC range calculated by the NCCLS median statistical method (3, 9) was 36 ± 3 mm (33 to 39 mm). However, only 94.8% of results were within this calculated range; therefore, 1 mm was added to the lower limit (32 mm), increasing the results in range to 99.2%. The *S. aureus* ATCC 25923 QC range (30 ± 3 mm) also calculated by the medians method had to be expanded by 1 mm at each extreme to provide ≥95% of results in the proposed QC range (26 to 34 mm). This was also true for the proposed QC range (30 to 38 mm) for *H. influenzae* ATCC 49766. The NCCLS median statistical method was applied without adjustments to propose QC ranges for *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *H. influenzae* ATCC 49247; the ranges were 30 to 36 mm (95.2% of results in range), 23 to 31 mm (99.4%), and 28 to 36 mm (99.2%), respectively. All six QC strains exhibited some variability of median values among the eight laboratories due to fuzzy zone diameter borders for

TABLE 3. Proposed QC ranges for NCCLS MIC and disk diffusion test methods[a]

QC organism	Proposed MIC range (μg/ml)	% Of results in range	Proposed disk diffusion range (mm)	% Of results in range
S. aureus ATCC 29213	0.25–1	100.0		
S. aureus ATCC 25923			26–34	98.8
E. faecalis ATCC 29212	0.06–0.5	100.0		
E. coli ATCC 25922	0.03–0.12	100.0	30–36	95.2
P. aeruginosa ATCC 27853	1–4	97.8	23–31	99.4
S. pneumoniae ATCC 49619	0.004–0.03	100.0	32–39	99.2
H. influenzae ATCC 49247	0.12–1	100.0	28–36	99.2
H. influenzae ATCC 49766	0.015–0.06	100.0	30–38	98.5

[a] Protocols designed using NCCLS guidelines (9–12).

BAL9141. It is suggested that all zone diameters be read at 100.0% or at complete inhibition of growth.

Table 3 summarizes all proposed QC ranges for the MIC and disk diffusion zone diameters for the QC strains tested. The proposed 3 or 4 $\log_2$ dilution ranges for the BAL9141 MIC results would encompass 97.8 to 100.0% of reported results. Similarly, the proposed 7- to 9-mm-zone-diameter ranges for the BAL9141 disk diffusion test would encompass 95.2 to 99.4% of the reported results.

This study summarizes results from a NCCLS-designed (9) collaborative study for establishing BAL9141 MIC QC ranges for the broth microdilution test method (11) and zone diameter QC ranges for the disk diffusion test (10). QC ranges for MIC and disk diffusion zone diameters that are proposed by this study will be important for the accurate phase 2 and 3 assessment of BAL9141, a novel broad-spectrum MRSA-active cephalosporin (2, 5, 7).

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REFERENCES

1. **Eliopoulos, G. M.** 2003. Quinupristin-dalfopristin and linezolid: evidence and opinion. Clin. Infect. Dis. **36:**473–481.
2. **Entenza, J. M., P. Hohl, I. Heinze-Krauss, M. P. Glauser, and P. Moreillon.** 2002. BAL9141, a novel extended-spectrum cephalosporin active against methicillin-resistant *Staphylococcus aureus* in treatment of experimental endocarditis. Antimicrob. Agents Chemother. **46:**171–177.
3. **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.
4. **Goldstein, F. W., and M. D. Kitzis.** 2003. Vancomycin-resistant *Staphylococcus aureus*: no apocalypse now. Clin. Microbiol. Infect. **9:**761–765.
5. **Hebeisen, P., I. Heinze-Krauss, P. Angehrn, P. Hohl, M. G. P. Page, and R. L. Then.** 2001. In vitro and in vivo properties of Ro 63–9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant Staphylococci. Antimicrob. Agents Chemother. **45:**825–836.
6. **Jones, R. N.** 2003. Global epidemiology of antimicrobial resistance among community-acquired and nosocomial pathogens: a five-year summary from the SENTRY antimicrobial surveillance program (1997–2001). Semin. Respir. Crit. Care Med. **24:**121–133.
7. **Jones, R. N., L. M. Deshpande, A. H. Mutnick, and D. J. Biedenbach.** 2002. In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci. J. Antimicrob. Chemother. **50:**915–932.
8. **Liu, C., and H. F. Chambers.** 2003. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob. Agents Chemother. **47:**3040–3045.
9. **National Committee for Clinical Laboratory Standards.** 2001. Development of in vitro susceptibility testing criteria and quality control parameters, 2nd ed. Document M23-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. **National Committee for Clinical Laboratory Standards.** 2003. Performance standards for antimicrobial disk susceptibility tests; approved standard, 8th ed. Document M2-A8. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 6th ed. Document M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing, 14th information supplement. Document M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.