

Determination of Quality Control Guidelines for MIC Dilution and Disk Diffusion Methods (NCCLS) When Testing LBM415, a Novel Peptide Deformylase Inhibitor

P923
ECCMID 2004

TR FRITSCHÉ, TR ANDEREGG, RN JONES
The JONES Group/JMI Laboratories, North Liberty, IA

Ronald N Jones, MD
ronald-jones@jmilabs.com
Tel: 319 665 3370
Fax: 319 665 3371

ABSTRACT

Background: Quality control (QC) guidelines remain necessary for accurate determination of antimicrobial susceptibility testing and should be established early in the development of new antimicrobial classes. LBM415 is a peptide deformylase (PDF) inhibitor rapidly progressing into Phase II and III human clinical trials, thus QC guidelines appear necessary for NCCLS methods. **Methods:** Multi-laboratory (7 or 8 sites) trials were initiated using the NCCLS M23-A2 guideline for QC determinations. Key technical details were: MIC phase - 4 Mueller-Hinton (MH) broth lots, 8 participant sites and 10 replicates of 4 appropriate QC strains; and disk diffusion phase - 3 MH agar lots, 7 sites and 10 replicates of 3 QC strains. Results were analyzed by statistical methods found in M23-A2. Control drugs included vancomycin, clarithromycin, linezolid and levofloxacin: 99.9 - 100.0% of control results were within published NCCLS ranges (640 and 1,050 results for MIC and zone tests, respectively). Inoculum concentration controls averaged 3.5×10^5 (MIC trial only). **Results:** Seven or 8 participants provided qualifying results in the 2 separate QC studies, and the calculated (proposed) ranges were (range; % results in range): *E. faecalis* ATCC 29212 (2-8 mg/L; 95.6), *S. aureus* ATCC 29213 (0.5-2 mg/L; 99.4), *S. pneumoniae* ATCC 49619 (0.25-1 mg/L; 97.5 and 30-37 mm; 97.6), *H. influenzae* ATCC 49247 (1-4 mg/L; 97.5 and 24-32 mm; 99.8), and *S. aureus* ATCC 25923 (25-35 mm; 97.8). All QC ranges were maximized to contain $\geq 95\%$ of reported results and zone size variation was elevated due to the bacteriostatic character of this PDF inhibitor, creating non-discreet zone edges. **Conclusions:** QC ranges for NCCLS methods when testing LBM415 have been established. Results from these NCCLS M23-A2-conforming trials can be utilized to control the accuracy of the susceptibility testing of this PDF inhibitor projected to be among the "first" to reach human clinical studies.

INTRODUCTION

- The emerging problems of bacterial resistance and co-resistance among the antimicrobial classes has led pharmaceutical companies to pursue novel targets and mechanisms of actions. One such target candidate is peptide deformylase (PDF), which is a required enzyme for prokaryote protein synthesis, but not essential for eukaryotic protein production. LBM415 is one of the first inhibitors of the bacterial PDF to be advanced into human clinical trials for the oral treatment of community-acquired respiratory tract and uncomplicated skin and skin-structure infections. This compound possesses potent activity against many antimicrobial-resistant gram-positive cocci, as well as having reasonable activity against *Haemophilus influenzae*, *Moraxella catarrhalis* and *Chlamydia pneumoniae*.
- To enable the accurate assessment of susceptibility test results in clinical trials, quality control (QC) guidelines for susceptibility testing methods must be established using recognized reference procedures, such as those promulgated by the National Committee of Clinical Laboratory Standards (NCCLS).
- This report summarizes the results of a multicenter study group that participated in establishing QC ranges for the MIC and disk diffusion methods when testing LBM415.

MATERIALS AND METHODS

- DETERMINATION OF MIC QC RANGES.** Frozen form and reference broth microdilution panels were prepared by TREK Diagnostics (Cleveland, OH, USA) and contained either 4 Mueller-Hinton broth lots supplemented, as needed, with 5% lysed horse blood, or with 4 lots of Haemophilus Test Medium (Difco, 2 lots; Hardy; BBL). Panels were stored at -80°C until used. Linezolid and clarithromycin served as internal control agents. Each of 8 laboratories tested 4 QC strains (including *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212 and *H. influenzae* ATCC 29247) daily for 10 days,

generating 320 MIC values per organism. Concurrent testing of the QC strains using linezolid as the internal control for *E. faecalis*, *S. aureus* and *S. pneumoniae*, and clarithromycin as the internal control for *H. influenzae*, showed that 100% of MIC results (640 values) were within published guidelines (NCCLS). Inoculum counts ranged from 3.0×10^4 to 1.0×10^6 CFU/mL (target inoculum, 5.0×10^5 CFU/mL).

- DETERMINATION OF DISK DIFFUSION QC RANGES.** This study used 3 lots of Mueller-Hinton agar supplemented with or without 5% sheep blood and 3 lots of Haemophilus Test Medium (HTM; Remel, Lenexa, KS; BBL, Sparks, MD, USA). Two different disk lots (Oxoid, Hampshire, UK; BBL, Sparks, MD, USA) and 1 disk lot of each internal control agent (vancomycin and levofloxacin; BBL) were used. Each laboratory tested 3 QC strains that included: *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 25923, and *H. influenzae* ATCC 49247. Experiments generated 2 zone diameter results on 3 media lots for 10 replicates with a total of 420 values for LBM415 tested against each QC strain. Concurrent testing of *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 25923 with vancomycin and levofloxacin, and *H. influenzae* ATCC 49247 with levofloxacin, produced 1050 QC results. Over 99.9% of internal QC results were within NCCLS published ranges.
- ANALYSIS.** The proposed QC ranges were optimized to contain $\geq 95\%$ of all reported results, as recommended by NCCLS M23-A2 guidelines.

RESULTS

MIC QC RANGES

- LBM415 MIC distributions when testing *S. pneumoniae* ATCC 49619 are shown in Table 1. The proposed QC MIC range was 0.25-1 mg/L, encompassing 97.5% of all reported results.
- Similar results were obtained for *H. influenzae* ATCC 49247 with nearly 69% of all QC results at the modal value of 2 mg/L. A MIC QC range of 1-4 mg/L containing 97.5% of all reported results was proposed.
- The results for *S. aureus* ATCC 29213 showed a modal value at 1 mg/L (71.9% of all results) and a proposed MIC range of 0.5-2 mg/L that would include 99.4% of all results.
- E. faecalis* ATCC 29212 had the highest percent of the total results (89.7%) at the MIC modal value of 4 mg/L. The proposed MIC QC range was 2-8 mg/L.
- The proposed MIC ranges for the 4 QC strains tested are summarized in Table 2. The proposed 3 log₂ dilution MIC ranges for LBM415 broth microdilution tests included 95.6-99.4% of all reported results in this initial QC study.

DISK DIFFUSION QC RANGES

- LBM415 zone diameter distributions among the 7 participating laboratories when testing *S. aureus* ATCC 25923 are shown in Table 3. With only 16.9% of the total results at the overall modal value (30 mm), the proposed QC range (25-35 mm) was 11 mm wide to achieve the $\geq 95\%$ of reported results.
- The overall modal value for *S. pneumoniae* ATCC 49619 was 33 mm (32.1% of total results; Table 4). The proposed QC range (30-37 mm) included the median (33) ± 3 mm as determined by NCCLS calculations plus an additional mm at the upper end of the range to achieve $\geq 95\%$ of results.
- The overall modal value for *H. influenzae* ATCC 49247 was 28 mm (21.2% of total results). The proposed QC range (24-32 mm) was calculated from the median method and included 99.8% of all of the laboratory results.
- All 3 QC strains exhibited variable modal values among participating laboratories, thus indicating some difficulty in reading zone diameter results. However, when results of zone diameters for the 2 lots of LBM415 disks and the 3 different media lots were compared, no significant lot-to-lot variation was observed for the 3 QC strains.
- The proposed zone diameter ranges for the 3 QC strains are summarized in Table 4. The 8 mm range for *S. pneumoniae* ATCC 49619, 11 mm range for *S. aureus* ATCC 25923 and 9 mm range for *H. influenzae* ATCC 49247 included 97.6-99.8% of reported disk diffusion zone diameter results.

CONCLUSIONS

- This study summarizes results from a NCCLS M23-A2 study design to establish LBM415 broth microdilution MIC and zone diameter QC ranges.
- With the emergence of antimicrobial resistance in clinically significant organisms, the need for newer antimicrobial agents with novel target sites of action becomes increasingly important. LBM415, a novel clinical candidate PDF inhibitor, has demonstrated excellent *in vitro* activity against the gram-positive and -negative organisms most frequently recovered from respiratory tract and skin and soft-tissue infections.
- The QC ranges for the MIC and disk diffusion methods proposed here will be important for the accurate and sustained development of this compound.

SELECTED REFERENCES

- Apfel CM, Locher H, Evers S, et al. *Antimicrob Agents Chemother* 2001;45:1058-64.
- Giglione C, Pierre M, Meinel T. *Mol Microbiol* 2000;36:1197-205.
- National Committee for Clinical Laboratory Standards. Approved guideline M23-A2. Wayne, PA; 2001.
- National Committee for Clinical Laboratory Standards. Approved standard M7-A6. Wayne, PA; 2003.
- National Committee for Clinical Laboratory Standards. 14th informational supplement M100-S14. Wayne, PA; 2004.
- Wise R, Andrews JM, Ashby J. *Antimicrob Agents Chemother* 2002;46:1117-8.

TABLE 1. Inter- and intra-laboratory comparisons of LBM415 MIC results for *S. pneumoniae* ATCC 49619

MIC (mg/L)	Occurrences by laboratory:								
	A	B	C	D	E	F	G	H	Total
0.12						8			8
0.25	4			20	14	31			69 ^a
0.5	36	26	22	20	26	1	29	18	178 ^a
1		14	18				11	22	65 ^a

a. Proposed MIC range that includes 97.5% of reported results.

TABLE 2. Distributions of LBM415 MIC values for all qualifying results from an 8-laboratory study using 4 control organisms

MIC (mg/L)	MIC occurrences by control strain:			
	<i>H. influenzae</i> ATCC 49247	<i>S. aureus</i> ATCC 29213	<i>E. faecalis</i> ATCC 29212	<i>S. pneumoniae</i> ATCC 49619
0.12				8
0.25		2		69 ^a
0.5	8	38 ^a		178 ^a
1	70 ^a	230 ^a		65 ^a
2	220 ^a	50 ^a	7 ^a	
4	22 ^a		287 ^a	
8			12 ^a	
>8			14	

a. Proposed ranges that included 95.6-99.4% of all reported MIC results.

TABLE 3. Inter- and intra-laboratory comparisons of LBM415 zone diameter (mm) results for *S. aureus* ATCC 25923

Zone diameter (mm)	Occurrences by laboratory:							Total
	A	B	C	D	E	F	G	
22			1					1
23			3		2			5
24			2		1			3
25			4		9			13 ^a
26			9		12		1	22 ^a
27			11		8	2	4	25 ^a
28	1	6	6		14	9	11	47 ^a
29	0	8	4		14	9	27	62 ^a
30	3	18	10	6		20	14	71 ^a
31	4	20	3	13		20	3	63 ^a
32	11	8	3	21				43 ^a
33	23		4	16				43 ^a
34	6			4				10 ^a
35	12							12 ^a

a. Proposed disk diffusion QC range that includes 97.8% of reported results.

TABLE 4. Distributions of LBM415 zone diameter (mm) values for all qualifying results from a 7-laboratory study using 3 control organisms

Zone diameter (mm)	QC organism zone diameter occurrences:		
	<i>S. aureus</i> ATCC 25923	<i>S. pneumoniae</i> ATCC 49619	<i>H. influenzae</i> ATCC 49247
22	1		
23	5		1
24	3		11 ^a
25	13 ^a		40 ^a
26	22 ^a		57 ^a
27	25 ^a		79 ^a
28	47 ^a		89 ^a
29	62 ^a		84 ^a
30	71 ^a	3 ^a	34 ^a
31	63 ^a	20 ^a	22 ^a
32	43 ^a	72 ^a	3 ^a
33	43 ^a	135 ^a	
34	10 ^a	84 ^a	
35	12 ^a	47 ^a	
36		30 ^a	
37		19 ^a	
38		8	
39		2	

a. Proposed ranges that include 97.6-99.8% of all reported disk diffusion results.