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Quality Control Guidelines for MIC Susceptibility Testing of Omiganan Pentahydrochloride (MBI 226), a Novel Antimicrobial Peptide

Antimicrobial cationic peptides are thought to be an important self-defense mechanism against infectious agents for plants, animals, and bacterial species (2). While cationic peptides vary in their mechanisms of action, most are reactive with bacterial cytoplasmic membranes (2). Omiganan pentahydrochloride (formerly MBI 226), a novel topical mammal-derived antimicrobial peptide (sequence: ILRWPWWPWRRK-amide), acts by disrupting the cytoplasmic membranes of both gram-positive and -negative bacteria, resulting in depolarization and cell death (D. Dugourd, C. Pasetka, D. Erfle, E. Rubinchik, K. Lee, and H. D. Friedland, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. A-47, 2002). Omiganan also demonstrated a dose-dependent inhibition of DNA, RNA, and protein synthesis at the macromolecular level, providing another mechanism of action (D. Dugourd, C. Pasetka, D. Erfle, E. Rubinchik, M. Guarna, and P. McNichol, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. A-46, 2002).

Omiganan is currently in phase III clinical trials as a prophylactic topical agent to minimize catheter-related bloodstream infections (D. J. Hoban, E. Witwicki, G. G. Zhanel, L. Palatnick, H. D. Friedland, and J. McBride, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-1647, 2002). The spectrum of activity for omiganan encompasses gram-positive bacteria and yeast. To determine the accurate assessment of the in vitro testing profiles of clinical isolates, quality control (QC) guidelines for omiganan are required (3).

A seven-laboratory consortium participated in a MIC QC study for omiganan by following the National Committee for Clinical Laboratory Standards (NCCLS)-recommended guidelines (3), test methods (4, 5), and interpretive criteria (6) and using common American Type Culture Collection (ATCC) QC strains. The reference frozen-form broth microdilution panels were prepared by TREK Diagnostics (Cleveland, Ohio) and contained three lots of cation-adjusted Mueller-Hinton broth (CA-MHB) (BBL, Sparks, Md.; Difco, Detroit, Mich.; Oxoid, Hampshire, England) supplemented or not with 5% lysed horse blood, three lots of cation-unadjusted Mueller-Hinton broth (MHB) (Oxoid [two lots] and Difco) supplemented or not with 5% lysed horse blood, and three lots of RPMI 1640 broth (Sigma [two lots], St. Louis, Mo.; Irvine Scientific, Santa Ana, Calif.). All panels were stored at −70°C until used. The omiganan standard powder was obtained from Micrologix Biotech, Inc. (Vancouver, Canada). The internal QC agents used in the study were levofloxacin (Ortho-McNeil, Rahway, N.J.) and vancomycin (Sigma Chemical) for the bacterial QC strains and flucytosine and amphotericin B from Sigma Chemical for the yeast QC strains. Each laboratory tested the following seven ATCC QC strains: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida krusei* ATCC 6258, and *Candida parapsilosis* ATCC 22019 over a 10-day period. In summary, each organism was tested once daily over 10 days in three medium lots by seven laboratories generating 210 ($10 \times 3 \times 7$) MIC results per QC strain.

Concurrent testing using vancomycin as the internal control for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619, using levofloxacin as the internal control for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, and using flucytosine and amphotericin B as the internal controls for *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 showed that 99.8% of all participant MIC results (1,399 values) were within published NCCLS guidelines (5). Inoculum colony counts were performed from the broth microdilution panels by subculturing in a quantitative manner onto drug-free plates. The inoculum counts for the bacterial QC testing ranged from 1.0×10^5 to 5.3×10^5 CFU/ml (average inoculum, 3.1×10^5 CFU/ml), and those for the yeast QC tests ranged from 5.5×10^2 to 4.5×10^3 CFU/ml (average inoculum, 2.1×10^3 CFU/ml).

Proposed QC ranges were optimized to encompass ≥95% of all results as recommended by the NCCLS M23-A2 guideline (3). MIC results for each tested antimicrobial agent were tabulated and compared by intra- and interlaboratory analysis and by medium lots. The bacterial QC strains were tested in CA-MHB and MHB, and these values were compared.

The results for *E. faecalis* ATCC 29212 did not show any shift due to the medium divalent cation differences (CA-MHB versus MHB). The modal value for both medium types was 64 μg/ml, with 75.5% of the total omiganan MIC results in CA-MHB and 90.0% of the total results in MHB achieving this value. The proposed omiganan MIC QC ranges for both CA-MHB and MHB were 32 to 128 μg/ml and encompassed 100% of all participant results. For *S. aureus* ATCC 29213, 57.1% of all results using CA-MHB and 58.1% of all results using MHB were at the modal value of 16 μg/ml. However, the proposed omiganan MIC QC ranges for CA-MHB (8 to 64 μg/ml) versus MHB (4 to 32 μg/ml) varied by 1 $\log_2$ dilution step (4 $\log_2$ dilution ranges).

S. pneumoniae ATCC 49619 and *E. coli* ATCC 25922 had similar medium-specific shifts, with MICs being 1 $\log_2$ dilution lower for the MHB than for CA-MHB. *S. pneumoniae* ATCC 49619 had a modal value of 64 μg/ml (75.7% of total results) in the CA-MHB versus a value of 32 μg/ml (92.4% of total results) in the MHB. Thus, the proposed 3 $\log_2$ dilution omiganan MIC QC range saw a twofold shift for CA-MHB (32 to 128 μg/ml) versus MHB (16 to 64 μg/ml). Both ranges included all of the reported results. *E. coli* ATCC 25922 had a modal value of 32 μg/ml (75.7% of total results) in the CA-MHB compared to a 16 μg/ml (80.5% of total results) in the MHB. The proposed omiganan MIC QC ranges also were 1 $\log_2$ dilution higher for CA-MHB when *E. coli* ATCC 25922 was used (Table 1).

P. aeruginosa ATCC 27853 QC trials exhibited a 2-$\log_2$-dilution difference in the modal omiganan values and proposed MIC QC ranges for CA-MHB and MHB. The modal value in CA-MHB was 128 μg/ml (86.7% of total results) compared to MICs in MHB of 32 μg/ml (60.0% of total results). The proposed omiganan MIC QC range for CA-MHB was 64 to 256 μg/ml, and that for MHB was 8 to 64 μg/ml. The cations in the media did not affect all QC strains in the same manner, so it is important to note the differences that medium selection makes for some QC strains, most notably *P. aeruginosa* ATCC 27853.

Table 1 also shows the distribution of omiganan MICs for the two yeast QC strains. A total of 49.5% of the results for *C. parapsilosis* ATCC 22019 were at the modal value of 64 μg/ml.

TABLE 1. Proposed MIC QC ranges for omiganan listed by medium type[a]

QC organism	Omiganan MIC (μg/ml) QC range (% in range) for:		
	CA-MHB	MHB	RPMI 1640
E. faecalis ATCC 29212	32–128 (100.0)	32–128 (100.0)	
S. aureus ATCC 29213	8–64 (99.5)	4–32 (100.0)	
S. pneumoniae ATCC 49619[b]	32–128 (100.0)	16–64 (100.0)	
E. coli ATCC 25922	16–64 (99.0)	8–32 (100.0)	
P. aeruginosa ATCC 27853	64–256 (100.0)	8–64 (100.0)	
C. parapsilosis ATCC 22019			32–128 (99.0)
C. krusei ATCC 6258			16–64 (100.0)

[a] RPMI 1640 was used for all tests of yeast.
[b] Lysed horse blood (2 to 5%) was added to CA-MHB for testing this QC strain.

The proposed omiganan MIC QC range of 32 to 128 μg/ml included 99.0% of all reported results. The modal value for *C. krusei* ATCC 6258 was 32 μg/ml (53.8% of results). The proposed omiganan MIC QC range of 16 to 64 μg/ml for *C. krusei* ATCC 6258 includes all reported results.

This study established QC results from a NCCLS M23-A2 (3) study design for omiganan tested by broth microdilution methods (4–6). Three $\log_2$ dilution ranges (mode $\pm$ 1 $\log_2$ dilution) were established for nine QC organism-medium ranges. Only on three occasions was it necessary to assign a 4-$\log_2$-dilution range, where nearly equal numbers of omiganan MICs occurred at two adjacent dilution steps (*S. aureus* ATCC 29213 in CA-MHB and MHB and *P. aeruginosa* ATCC 27853 in MHB). As omiganan advances through phase III clinical trials and beyond, the MIC QC ranges established during this study will permit accurate laboratory susceptibility testing as an aid in assessing the value of the compound against contemporary cutaneous bacterial isolates or for detection of emerging resistances as part of local or regional epidemiology programs. The latter applications would be similar to those currently used in in vitro tests of mupirocin, another topical agent, to confirm resistances in clinically refractory strains or to select topical agents for formulary addition (1).

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