# Quality Control Ranges for Performance Assessment of a Revised Broth Microdilution Susceptibility Testing Method for Telavancin

James E. Ross, Ronald N. Jones, Helio S. Sader, David J. Farrell
JMI Laboratories, North Liberty, IA, USA

**Contact information:**
James Ross
JMI Laboratories
345 Beaver Kreek Ctr, Ste A
North Liberty, Iowa, 52317, USA
Phone: 319-665-3370
Fax: 319-665-3371
jim-ross@jmilabs.com

## ABSTRACT

**Background.** The broth microdilution (BMD) method for telavancin MIC determination was revised to utilize dimethyl sulfoxide (DMSO) as solvent for stock solution preparation and as stock solution diluent for panel production, following the CLSI guidelines for water-insoluble agents. The revised method also incorporates polysorbate-80 (P-80; or Tween-80; 0.002%) in the MIC test medium. Like other lipoglycopeptides, addition of P-80 was deemed necessary for more accurate and reproducible telavancin MIC determinations. This study aimed to re-establish MIC quality control (QC) ranges for telavancin when using this revised BMD method.

**Materials.** This study included eight laboratories compliant with the CLSI M23-A3 guidelines. Frozen-form BMD MIC panels were produced according to the revised method described above under Good Manufacturing Practices. Four media lots (from three manufacturers) of cation-adjusted Mueller-Hinton broth (CA-MHB; with 2–5% lysed horse blood for *Streptococcus pneumoniae*) were utilized. Telavancin was tested against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619 QC strains. Ten replicate tests were performed for each QC strain generating 320 BMD MIC values/strain. Vancomycin (control) was tested according to CLSI guidelines and all MIC results were within published ranges.

**Results.** The table lists the MIC QC ranges for telavancin obtained by the revised BMD method and those previously established. Telavancin modal MIC values (% of total) obtained by the revised BMD method for *S. aureus*, *E. faecalis* and *S. pneumoniae* were 0.06 (71.3%), 0.06 (82.8%) and 0.008 µg/mL (83.8%), respectively. The telavancin MIC QC ranges obtained by the revised BMD method for *S. aureus* and *E. faecalis* were lower than the previously established method. All three revised MIC QC ranges consisted of only three doubling dilution steps. Medium lots exhibited only minor variations of MIC distributions for telavancin results.

**Conclusion.** These re-established MIC QC ranges for assessing the performance of the revised BMD method for telavancin replaced those published in the CLSI M100-S23 and earlier documents. These MIC QC ranges apply when using the revised method for telavancin, which can be utilized with updated MIC interpretive breakpoints established by the Food and Drug Administration (FDA).

| QC organism (ATCC) | Telavancin MIC (µg/mL) QC ranges for BMD (% in range) | |
|---|---|---|
| | Revised | Previous |
| *S. aureus* (ATCC 29213) | 0.03–0.12 (100.0) | 0.12–1 |
| *E. faecalis* (ATCC 29212) | 0.03–0.12 (100.0) | 0.12–0.5 |
| *S. pneumoniae* (ATCC 49619) | 0.004–0.015 (100.0) | 0.004–0.03 |

## INTRODUCTION

- Telavancin is a once-daily parenteral semi-synthetic lipoglycopeptide agent approved in the United States and Canada for the treatment of adult patients with complicated skin and skin structure infections (cSSSI) caused by susceptible Gram-positive pathogens.[1]
- Telavancin was also approved in the United States and Europe for the treatment of adult patients with hospital-acquired bacterial pneumonia, including ventilator-associated bacterial pneumonia (HABP/VABP) due to susceptible isolates of *Staphylococcus aureus* (methicillin-resistant strains [MRSA] only in Europe), when alternative medicines are unsuitable.[2,3]
- Telavancin is active and bactericidal against nearly all clinically important Gram-positive bacteria: staphylococci (including methicillin-resistant and vancomycin-intermediate strains), streptococci (including multidrug-resistant pneumococci), enterococci, Gram-positive anaerobes such as Clostridia (including *Clostridium difficile*), and other less commonly encountered Gram-positive pathogens.[2,3]
- Previous Clinical and Laboratory Standards Institute (CLSI) documents (M100-S15 through M100-S23) recommended the use of dimethyl sulfoxide (DMSO) as solvent for preparation of stock solution, and water as stock solution diluent for preparing 96-well frozen-form panels for telavancin susceptibility testing.[4,5]

- This method was used to establish the previous telavancin minimum inhibitory concentration (MIC) quality control (QC) ranges.[4,5] However, the broth microdilution (BMD) method for telavancin was revised, and now consists of using DMSO as solvent and diluent for stock solution preparation and dilution, following the CLSI guidelines for stock solution and dilution preparations of water-insoluble agents. This modification was shown to improve drug solubility (see Poster #2567 for further details).
- In addition, polysorbate-80 (P-80) was added to the Mueller-Hinton (MH) test medium at a final concentration of 0.002%. Like other lipoglycopeptides, addition of P-80 was deemed necessary for more accurate and reproducible telavancin MIC determinations.
- Studies conducted during the development of the revised method demonstrated that the MIC results for telavancin when tested against staphylococci and enterococci were four- to eight-fold lower than those obtained by the previous established BMD method.
- Therefore, this study was conducted to re-establish the MIC QC ranges for telavancin when utilizing the revised BMD method.

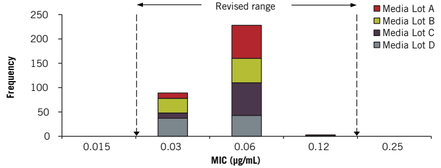
## MATERIALS AND METHODS

- The study presented here was performed according to the guidelines found in the CLSI M23-A3 (2008) document, which specifies the use of at least seven laboratories and three different manufacturers of media. This study utilized eight laboratories and four different media lots.
- BMD panels included four cation-adjusted MH broth media lots produced by Difco Laboratories (Detroit, MI, USA), Becton Dickinson (BD) (Sparks, MD, USA), and Oxoid (Hampshire, UK).
- Telavancin powder was provided by Theravance, Inc. (South San Francisco, CA, USA) and vancomycin (internal control) was acquired from Sigma-Aldrich (St. Louis, MO, USA). Telavancin stock solution was dissolved and diluted in DMSO following the CLSI (Table 8B; M100-S24, 2014) recommendations for water-insoluble agents for the preparation of frozen-form panels according to the revised method. P-80 was added to the MH test medium at a final concentration of 0.002%.[1,6,7]
- Panels were manufactured by ThermoFisher Scientific (formerly TREK Diagnostics Systems/Sensititre™; Cleveland, OH, USA), a Good Manufacturing Process facility, under direct observation of JMI Laboratories personnel (see Poster #2567 for additional information).
- Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates.

## RESULTS

- The telavancin MIC ranges obtained against *S. aureus* American Type Culture Collection (ATCC) 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619 QC strains by the revised and previously established BMD methods are summarized in **Table 1**.
- Telavancin displayed MIC results between 0.03 and 0.12 µg/mL, with an overall modal MIC value (71.3% of results) of 0.06 µg/mL when tested against *S. aureus* ATCC 29213 by the revised method (**Table 1** and **Table 2; Figure 1**). Additional data analysis was performed using the Range Finder program, which confirmed the modal MIC and proposed a QC range of 0.03–0.12 µg/mL.[8]
- The telavancin modal MIC (0.25 µg/mL; 62.9% of values) and MIC range (0.12–1 µg/mL) obtained against *S. aureus* ATCC 29213 by the previously established method were, respectively, four-fold higher and one doubling dilution wider than those obtained by the revised method (**Table 1**).
- Telavancin MIC results obtained against the *E. faecalis* ATCC 29212 strain by the revised method were within a three $log_2$ dilution range (0.03–0.12 µg/mL) (**Table 1** and **Table 2**; **Figure 2**) with a modal MIC value of 0.06 µg/mL; results were confirmed by the Range Finder program.
- *S. pneumoniae* ATCC 49619 had telavancin MIC results within 0.004 to 0.015 µg/mL (**Table 1** and **Table 2**; **Figure 3**). These telavancin MIC results obtained against *S. pneumoniae* ATCC 49619 with the revised method suggested a QC range similar to that previously established (0.004–0.03 µg/mL). However, a greater reproducibility (83.8% vs 48.3% of MIC values at 0.008 µg/mL obtained by the revised and previous methods, respectively) and narrower range was obtained using the revised methodology.
- All vancomycin results were within published QC ranges providing a valid internal control.
- All broth medium lots and laboratories shared the same modal MIC value, regardless of the ATCC strain tested (except for Laboratory B vs *S. aureus* ATCC 29213, where the modal MIC was 0.03 µg/mL, compared to 0.06 µg/mL for the remaining seven laboratories) (**Table 2**).

**Figure 1.** Telavancin MIC distributions for *S. aureus* ATCC 29213



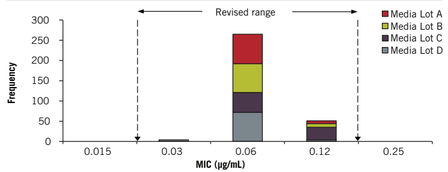

ATCC = American Type Culture Collection; MIC = minimum inhibitory concentration.

**Table 1.** Quality control MIC ranges for telavancin when tested against ATCC strains using the previous and revised broth microdilution methods

| QC Organism | MIC (µg/mL) | Number (%) of isolates inhibited at telavancin MIC (µg/mL) by testing method[a] | |
|---|---|---|---|
| | | Revised[b] | Previous[c] |
| *S. aureus* ATCC 29213 | 1 | – | 17 (7.1) |
| | 0.5 | – | 71 (29.6) |
| | 0.25 | – | 151 (62.9) |
| | 0.12 | 3 (0.9) | 1 (0.4) |
| | 0.06 | 228 (71.3) | – |
| | 0.03 | 89 (27.8) | – |
| *E. faecalis* ATCC 29212 | 1 | – | 1 (0.4) |
| | 0.5 | – | 34 (14.2) |
| | 0.25 | – | 203 (84.6) |
| | 0.12 | 51 (15.9) | 2 (0.8) |
| | 0.06 | 265 (82.8) | – |
| | 0.03 | 4 (1.3) | – |
| *S. pneumoniae* ATCC 49619 | 0.03 | – | 1 (0.4) |
| | 0.015 | 45 (14.1) | 22 (9.2) |
| | 0.008 | 268 (83.8) | 116 (48.3) |
| | 0.004 | 7 (2.2) | 101 (42.1) |
| | 0.002 | – | – |

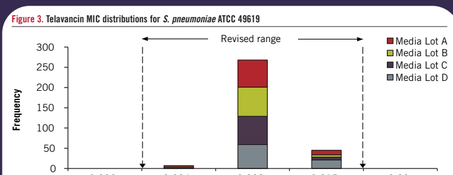
[a] Boxed results represent the MIC QC ranges for the revised and previous susceptibility testing methods approved by CLSI and published in the M100-S24, and M100-S15 through M100-S23 documents, respectively.
[b] MIC QC ranges for the revised method proposed and approved by CLSI (published in M100-S24). All values within ranges.
[c] MIC QC ranges proposed for the previous method were as follows: *S. aureus* ATCC 29213 (0.12–1 µg/mL; all MIC values within range), *E. faecalis* ATCC 29212 (0.12–0.5 µg/mL; 99.6% of MIC values within range), and *S. pneumoniae* ATCC 49619 (0.002–0.015 µg/mL; 99.6% of MIC values within range).
ATCC = American Type Culture Collection; MIC = minimum inhibitory concentration; QC = quality control.

**Figure 2.** Telavancin MIC distributions for *E. faecalis* ATCC 29212




ATCC = American Type Culture Collection; MIC = minimum inhibitory concentration.

**Figure 3.** Telavancin MIC distributions for *S. pneumoniae* ATCC 49619




ATCC = American Type Culture Collection; MIC = minimum inhibitory concentration.

**Table 2.** Medium lot, and inter- and intra-laboratory comparisons of telavancin MIC results obtained when tested against the listed ATCC QC strains

| ATCC strain/MIC (µg/mL) | Occurrences by medium lot | | | | Occurrences by laboratory | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | E | F | G | H | |
| *S. aureus* 29213 | | | | | | | | | | | | | |
| 0.03 | 11 | 30 | 11 | 37 | 12 | 27 | – | 7 | 8 | 8 | 16 | 11 | 89 |
| 0.06 | 68 | 50 | 67 | 43 | 28 | 12 | 40 | 33 | 31 | 31 | 24 | 29 | 228 |
| 0.12 | 1 | – | 2 | – | – | 1 | – | – | 1 | 1 | – | – | 3 |
| Total | 80 | 80 | 80 | 80 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 320 |
| Mode | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.03 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Geomean | 0.06 | 0.05 | 0.06 | 0.04 | 0.05 | 0.04 | 0.06 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| $Log_2$ dilution range | 3 | 2 | 3 | 2 | 2 | 3 | 1 | 2 | 3 | 3 | 2 | 2 | 3 |
| *E. faecalis* 29212 | | | | | | | | | | | | | |
| 0.03 | – | – | – | 4 | – | 2 | – | 1 | – | – | – | 1 | 4 |
| 0.06 | 73 | 71 | 49 | 72 | 38 | 37 | 37 | 31 | 35 | 23 | 28 | 36 | 265 |
| 0.12 | 7 | 9 | 31 | 4 | 2 | 1 | 3 | 8 | 5 | 17 | 12 | 3 | 51 |
| Total | 80 | 80 | 80 | 80 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 320 |
| Mode | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Geomean | 0.06 | 0.06 | 0.08 | 0.06 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.08 | 0.07 | 0.06 | 0.07 |
| $Log_2$ dilution range | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 3 |
| *S. pneumoniae* 49619 | | | | | | | | | | | | | |
| 0.004 | 2 | 2 | 3 | – | – | 1 | 6 | – | – | – | – | – | 7 |
| 0.008 | 67 | 72 | 70 | 59 | 23 | 33 | 29 | 34 | 33 | 40 | 40 | 36 | 268 |
| 0.015 | 11 | 6 | 7 | 21 | 17 | 6 | 5 | 6 | 7 | – | – | 4 | 45 |
| Total | 80 | 80 | 80 | 80 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 320 |
| Mode | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| Geomean | 0.009 | 0.008 | 0.008 | 0.010 | 0.011 | 0.009 | 0.008 | 0.009 | 0.009 | 0.008 | 0.008 | 0.009 | 0.009 |
| $Log_2$ dilution range | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 2 | 2 | 1 | 1 | 2 | 3 |

ATCC = American Type Culture Collection; MIC = minimum inhibitory concentration; QC = quality control.

## CONCLUSIONS

- Overall, the MIC QC ranges obtained by the revised BMD method for telavancin described in this study are narrower and more reproducible when compared with those obtained by the previously established susceptibility testing method.
- These revised telavancin QC ranges were approved by CLSI and published in the M100-S24 (2014) document. In addition, an updated telavancin product package insert was approved by the US Food and Drug Administration (February 2014), which contains the revised testing method, the new QC MIC ranges as presented here, as well as the new interpretive breakpoints, which correlate to these revised methods.[1]

## REFERENCES

1. VIBATIV® Package Insert 2014. Available at http://www.vibativ.com Accessed 2 April 2014.
2. Putnam SD et al. *Diagn Microbiol Infect Dis* 2010;67:359-368.
3. Mendes RE et al. *Antimicrob Agents Chemother* 2012;56:3999-4004.
4. National Committee for Clinical Laboratory Standards 2004. Subcommittee on antimicrobial susceptibility testing - Book II. Tempe, AZ, USA.
5. Clinical and Laboratory Standards Institute 2013. *M100-S23. 23rd Informational Supplement.* Wayne, PA, USA.
6. Clinical and Laboratory Standards Institute 2012. *M07-A9. Approved Standard: Ninth edition.* Wayne, PA, USA.
7. Clinical and Laboratory Standards Institute 2014. *M100-S24. 24th Informational Supplement.* Wayne, PA, USA.
8. Turnidge J et al. *Antimicrob Agents Chemother* 2007;51:2483-2488.

Presented at the 114th General Meeting of the American Society for Microbiology (ASM); May 17–20, 2014; Boston, MA, USA.

The research and publication process was supported by Theravance, Inc. Poster development support was provided by Suzanne Douthwaite, a medical writer at Envision Scientific Solutions, funded by Theravance, Inc.