A-001

# Medium Quality and Susceptibility Testing Results of Telavancin Using CLSI Standardized Methods (M2-A9 and M7-A7)

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## ABSTRACT

**Background:**
Telavancin (TLV), a novel lipoglycopeptide, has potent bactericidal activity against clinically important Gram-positive cocci including MRSA and other resistant (R) species. To effectively utilize this potentially valuable agent, susceptibility (S) tests should be accurate and reproducible. Preliminary QC ranging studies on structurally similar agents have indicated possible adverse medium influences. This study was undertaken to determine effects of medium quality on TLV reference S testing.

**Methods:**
The study was designed to determine variation in disk zone diameters (mm) and MIC values (µg/ml) produced by 10 Mueller-Hinton (MH) agar lots (5 manufacturers) with 2 TLV disk lots, and 5 MH broth lots (5 manufacturers), respectively. All testing was performed in replicate over several days (9 or 10 organisms; 270-300 and 600 replicates for MIC and disk tests, respectively). Vancomycin (VAN) was used as a control glycopeptide with results compared to CLSI QC ranges for control strains

**Results:**
No significant zone differences (0.07 mm variance) between telavancin disk lots were observed. Some MH agar lots showed inter-manufacturer and –lot variations, however >97% of all QC results remained within proposed QC ranges. An example of agar medium lot induced variance follows in the table (10 lots; 60 tests for *S. aureus* ATCC 25923 [QC range = 16-20 mm]). For broth lots, MIC results were highly correlative (≤ 1 $\log_2$ dilution step) between medium lots and producers.

| | Zone replicate occurrences by lot (coded, 1 and 2) and medium producer (coded, A-E): | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | | B | | C | | D | | E | |
| Zone (mm) | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 15 | - | - | 3 | - | - | - | - | - | - | - |
| 16 | 3 | 1 | 3 | 5 | - | - | 3 | - | - | - |
| 17 | 1 | 3 | - | 1 | 1 | - | 3 | 6 | 3 | 3 |
| 18 | 2 | 2 | - | - | 4 | 4 | - | - | 3 | 3 |
| 19 | - | - | - | - | 1 | 2 | - | - | - | - |
| 20 | - | - | - | - | - | - | - | - | - | - |

**Conclusions:**
Variations in CLSI based testing results for TLV were observed between some agar or broth medium manufacturers and lots. Poor growth of certain enterococcal isolates with some MH agar lots appeared to influence TLV disk testing results (falsely large zones). Disk and broth QC ranges for TLV appear robust and performed well in this study. The results of this study indicate that out of range QC values might be attributable to medium quality variations; high quality media is critical for susceptibility testing of TLV.

## INTRODUCTION

Telavancin (Figure 1), a novel lipoglycopeptide, is a rapidly bactericidal antimicrobial agent and is highly active against Gram-positive bacteria, including strains of methicillin-resistant *Staphylococcus aureus* (MRSA), and some vancomycin-non-susceptible *S. aureus* (VISA or VRSA). Telavancin has concentration-dependant killing and multiple mechanisms of action via transglycosylase activity leading to the inhibition of cell-wall synthesis and interactions with the bacterial membrane. These interactions dissipate the membrane potential and changing cell permeability that correlates with loss in bacterial viability. The serum elimination half-life ranges from 7 to 9 hours and produces a long post-antibiotic effect. These characteristics led to once daily dosing trials for indications such as complicated skin and soft tissue infections and hospital acquired pneumonia.

Initial in vitro potency studies and subsequent determinations of quality control (QC) ranges per CLSI M23-A2 (2001) guidelines noted possible medium-dependent MIC or zone diameter variations (CMI, Tualatin, OR). This investigation studies Mueller-Hinton agar and broth microdilution lots (1 or 2 lots/manufacturer) from five different producers and uses multiple replicate tests of QC and wildtype (WT) Gram-positive strains.

## MATERIALS AND METHODS

The study design was implemented by one laboratory using the following components:

1. Broth phase tested telavancin and the vancomycin control in five different Mueller-Hinton broth lots, one or two from four manufacturers (BBL [2 lots], Difco, Oxoid and Criterion);
2. Agar phase tested telavancin disks (two lots produced by BBL [#3336991] and Hardy [#149385] and 30-µg vancomycin disk controls (also two lots). Ten agar lots were used, two each from five different manufacturers (BBL, Difco, Accumedia, Oxoid and Criterion).

All procedures followed Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) documents M2-A9 and M7-A7 (2006). QC ranges for both agents were derived from CLSI M100-S17 (2007).

Organisms tested by MIC methods were QC strains *S. aureus* ATCC 25923 and 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. Replicate results were presented as mean zones (mm) or MIC values (µg/ml) and were compared to the published ranges in M100-S17. Clinical strains included WT *S. aureus* (two strains, one MRSA), coagulase-negative staphylococci (CoNS; two strains), and one strain each of *E. faecalis*, *E. faecium* and *S. pneumoniae* (300 telavancin MIC results were generated). For agar disk diffusion tests, three replicates of two QC strains and seven WT strains were tested for a total of 600 zone diameters. Equal numbers of vancomycin values were produced.

## RESULTS

- Telavancin and vancomycin disk lots (BBL and Hardy) performed acceptably regardless of medium lot, thus producing all zones within ±1 mm and 59-61% of zones being identical.
- Replicate disk diffusion tests of QC strains showed that one lot of agar (Difco, Table 1) produced out-of-range zone diameter results with vancomycin when using *S. aureus* ATCC 25923.
- Vancomycin disk diffusion QC results for *S. pneumoniae* also showed wide inter-lot variations and two lots from the same manufacturer (Accumedia) produced unacceptable (large) zones (data not shown). Notably, telavancin QC results with the same strain (Table 2) and same media lots were also significantly different.
- WT strain testing by disk diffusion showed medium-specific telavancin zone diameter differences as follows:
  - larger zones with CoNS on Criterion agar lots (also inter-lot variation)
  - large zones when testing *E. faecium* on Accumedia lots, and between lot variations for Criterion media.
- Broth microdilution MIC results for vancomycin (not shown) and telavancin demonstrated greater uniformity across five lots (four manufacturers). Only one lot (Oxoid) was associated with a one doubling dilution lower MIC result for the *S. aureus* ATCC 29213 QC strain (Table 3). In general, that lot also had a trend toward lower telavancin and vancomycin MIC values for all tested species groups.

**Figure 1.** Structure of telavancin.

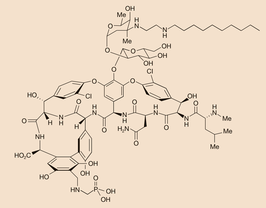

**Table 1.** Comparisons of telavancin disk diffusion results for 10 lots of Mueller-Hinton agar tested in replicate (six results/lot) against *S. aureus* ATCC 25923.

| | BBL | | Difco | | Accumedia | | Oxoid | | Criterion | |
|---|---|---|---|---|---|---|---|---|---|---|
| Zone diameter (mm) | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 14 | | | | | | | | | | |
| 15 | | | 3 | | | | | | | |
| 16[a] | 3 | 1 | 3 | 5 | | | 3 | | | |
| 17[a] | 1 | 3 | | 1 | 1 | | 3 | 6 | 3 | 3 |
| 18[a] | 2 | 2 | | | 4 | 4 | | | 3 | 3 |
| 19[a] | | | | | 1 | 2 | | | | |
| 20[a] | | | | | | | | | | |
| 21 | | | | | | | | | | |

a. Approved CLSI QC range [M100-S17, 2007].

**Table 2.** Mean zone diameters for telavancin using two disk lots placed on ten different Mueller-Hinton medium lots (five manufacturers).

| | Mean zone diameter (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BBL | | Difco | | Accumedia | | Oxoid | | Criterion | |
| Organism | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| QC strains | | | | | | | | | | |
| *S. aureus* ATCC 25923[a] | 16.8 | 17.2 | 15.5[c] | 16.2[c] | 18.0 | 18.3 | 16.5 | 17.0 | 17.5 | 17.5 |
| *S. pneumoniae* ATCC 49619[a] | 19.8 | 19.7 | 20.7 | 21.3 | 22.8[e] | 22.7[e] | 19.0 | 19.3 | 22.3 | 21.5 |
| WT strains | | | | | | | | | | |
| *S. aureus*[b] | 17.2 | 17.3 | 15.8 | 16.2 | 18.1 | 18.1 | 16.0 | 16.2 | 17.3 | 17.2 |
| CoNS[c] | 20.8 | 20.5 | 21.5 | 20.9 | 21.8 | 22.0 | 20.7 | 20.8 | 23.8[d,e] | 22.5[d,e] |
| *S. pneumoniae*[c] | 19.9 | 20.1 | 20.5 | 21.0 | 22.2[e] | 22.6[e] | 19.0 | 19.4 | 21.8 | 21.0 |
| *E. faecalis*[c] | 17.0 | 16.0 | 16.5 | 17.0 | 18.0 | 18.0 | 16.0 | 16.5 | 17.5 | 18.0 |
| *E. faecium*[3] | 22.0 | 21.0 | 21.0 | 21.0 | 26.0[e] | 26.5[e] | 20.0 | 20.0 | 23.0[c] | 24.5[d] |

a. Six replicates/lot.
b. 24 replicates/lot.
c. 12 replicates/lot.
d. Significant variations between lot from same manufacturers (>1 mm).
e. Significant variations between manufacturers.

**Table 3.** Mean MIC values when replicate testing of telavancin using five lots of Mueller-Hinton broth.

| | Mean MIC (µg/ml) by manufacturer and lot: | | | | |
|---|---|---|---|---|---|
| Organism | BBL-1 | BBL-2 | Criterion | Difco | Oxoid |
| QC strains[a] | | | | | |
| *S. aureus* ATCC 29213 | 0.25 | 0.25 | 0.25 | 0.25 | 0.12[b] |
| *E. faecalis* ATCC 29212 | 0.25 | 0.42 | 0.25 | 0.25 | 0.17 |
| *S. pneumoniae* ATCC 49619 | 0.008 | 0.012 | 0.012 | 0.012 | 0.008 |
| WT strains[a] | | | | | |
| Staphylococci and enterococci | 0.25 | 0.31 | 0.27 | 0.25 | 0.15 |
| *S. pneumoniae* | 0.012 | 0.012 | 0.014 | 0.012 | 0.012 |

a. Three replicates per strain per medium lot.
b. Significant variation in MIC (≥one $\log_2$ dilutions).

## CONCLUSIONS

- Some Mueller-Hinton agar medium lots (Accumedia, Criterion, Difco) can alter telavancin zone diameter results for CoNS, *E. faecium* and pneumococcal wildtype strains although QC tests may remain within CLSI-published limits. Vancomycin zones were also affected, but to a lesser degree.
- Mueller-Hinton broth medium lots (Oxoid) showed only minor telavancin MIC variation (≤one $\log_2$ dilution for QC strains).
- Overall, these results did not detect any systematic problem with telavancin in vitro testing. However, extreme care must be exercised to select high quality agar and broth lots that conform to QC standards published by the CLSI (M100-S17), and additionally, support the adequate growth of other routinely tested Gram-positive pathogens such as CoNS and non-*E. faecalis* enterococcal species.

## SELECTED REFERENCES

1. Barcia-Macay M, Lemaire S, Mingeot-Leclercq MP, Tulkens PM, Van Bambeke F (2006). Evaluation of the extracellular and intracellular activities (human THP-1 macrophages) of telavancin versus vancomycin against methicillin-susceptible, methicillin-resistant, vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 58: 1177-1184.
2. Barrett JF (2005). Recent developments in glycopeptide antibacterials. *Curr Opin Investig Drugs* 6: 781-790.
3. Barriere S, Genter F, Spencer E, Kitt M, Hoelscher D, Morganroth J (2004). Effects of a new antibacterial, telavancin, on cardiac repolarization (QTc interval duration) in healthy subjects. *J Clin Pharmacol* 44: 689-695.
4. Clinical and Laboratory Standards Institute. (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition*. Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute. (2007). *M100-S17, Performance standards for antimicrobial susceptibility testing, 17th informational supplement*. Wayne, PA: CLSI.
6. Clinical and Laboratory Standards Institute. (2006). *M2-A9. Performance standards for antimicrobial disk susceptibility tests; approved standard - ninth edition*. Wayne, PA: CLSI.
7. Gander S, Kinnaird A, Finch R (2005). Telavancin: In vitro activity against staphylococci in a biofilm model. *J Antimicrob Chemother* 56: 337-343.
8. Goldstein EJ, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT (2004). In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic Gram-positive species and *Corynebacterium* spp. *Antimicrob Agents Chemother* 48: 2149-2152.
9. Hegde SS, Reyes N, Wiens T, Vanasse N, Skinner R, McCullough J, Kaniga K, Pace J, Thomas R, Shaw JP, Obedencio G, Judice JK (2004). Pharmacodynamics of telavancin (TD-6424), a novel bactericidal agent, against Gram-positive bacteria. *Antimicrob Agents Chemother* 48: 3043-3050.
10. Higgins DL, Chang R, Debabov DV, Leung J, Wu T, Krause KM, Sandvik E, Hubbard JM, Kaniga K, Schmidt DE, Jr., Gao Q, Cass RT, Karr DE, Benton BM, Humphrey PP (2005). Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 49: 1127-1134.
11. King A, Phillips I, Kaniga K (2004). Comparative in vitro activity of telavancin (TD-6424), a rapidly bactericidal, concentration-dependent anti-infective with multiple mechanisms of action against Gram-positive bacteria. *J Antimicrob Chemother* 53: 797-803.
12. Leuthner KD, Cheung CM, Rybak MJ (2006). Comparative activity of the new lipoglycopeptide telavancin in the presence and absence of serum against 50 glycopeptide non-susceptible staphylococci and three vancomycin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 58: 338-343.
13. Madrigal AG, Basuino L, Chambers HF (2005). Efficacy of Telavancin in a rabbit model of aortic valve endocarditis due to methicillin-resistant *Staphylococcus aureus* or vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 49: 3163-3165.
14. National Committee for Clinical Laboratory Standards. (2001). *Approved guideline M23-A2: Development of in vitro susceptibility testing criteria and quality controls parameters, 2nd ed*. Wayne, PA: NCCLS.
15. Pace JL, Krause K, Johnston D, Debabov D, Wu T, Farrington L, Lane C, Higgins DL, Christensen B, Judice JK, Kaniga K (2003). In vitro activity of TD-6424 against *Staphylococcus aureus*. *Antimicrob Agents Chemother* 47: 3602-3604.
16. Reyes N, Skinner R, Kaniga K, Krause KM, Shelton J, Obedencio GP, Gough A, Conner M, Hegde SS (2005). Efficacy of telavancin (TD-6424), a rapidly bactericidal lipoglycopeptide with multiple mechanisms of action, in a murine model of pneumonia induced by methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 49: 4344-4346.
17. Reyes N, Skinner R, Benton BM, Krause KM, Shelton J, Obedencio GP, Hegde SS (2006). Efficacy of telavancin in a murine model of bacteraemia induced by methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 58: 462-465.
18. Shaw JP, Seroogy J, Kaniga K, Higgins DL, Kitt M, Barriere S (2005). Pharmacokinetics, serum inhibitory and bactericidal activity, and safety of telavancin in healthy subjects. *Antimicrob Agents Chemother* 49: 195-201.
19. Stryjewski ME, O'Riordan WD, Lau WK, Pien FD, Dunbar LM, Vallee M, Fowler VG, Jr., Chu VH, Spencer E, Barriere SL, Kitt MM, Cabell CH, Corey GR (2005). Telavancin versus standard therapy for treatment of complicated skin and soft-tissue infections due to Gram-positive bacteria. *Clin Infect Dis* 40: 1601-1607.
20. Stryjewski ME, Chu VH, O'Riordan WD, Warren BL, Dunbar LM, Young DM, Vallee M, Fowler VG, Jr., Morganroth J, Barriere SL, Kitt MM, Corey GR (2006). Telavancin versus standard therapy for treatment of complicated skin and skin structure infections caused by Gram-positive bacteria: FAST 2 study. *Antimicrob Agents Chemother* 50: 862-867.
21. Stucki A, Gerber P, Acosta F, Cottagnoud M, Cottagnoud P (2006). Efficacy of telavancin against penicillin-resistant pneumococci and *Staphylococcus aureus* in a rabbit meningitis model and determination of kinetic parameters. *Antimicrob Agents Chemother* 50: 770-773.