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Dalbavancin MIC Testing: Validation and Test Reproducibility of Commercial Reagents

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ABSTRACT

Background: Dalbavancin (DAL), formerly BI397, is a glycopeptide having an extended serum $T_{1/2}$ that allows once-weekly dosing intervals. As this agent with potent Gram-positive activity advances into Phase III clinical trials, reliable MIC methods and quality control guidelines will be required.

Methods: The validation was performed by NCCLS M23-A2 guidelines to determine MIC reproducibility (≥ 10 organisms x 3 tests/day x 3 days = ≥ 90 tests) and comparative MIC accuracy to the reference NCCLS MIC (REF; M7-A6, 2003) using ≥ 100 strains representing the following organism groups: staphylococci, enterococci, *Streptococcus pneumoniae*, other non-*S. pneumoniae* streptococci and selected species resistant to the studied glycopeptides. The MIC panels were manufactured by Sensititre (TREK Diagnostics, Cleveland, OH).

Results: Validation phase test results comparing dry-form MICs to REF MICs were (% identical/%±2-fold/%±4-fold): for staphylococci (65/99/100%), for enterococci (51/100/100%), for *S. pneumoniae* (85/100/100%), for other streptococcal species (92/100/100%) and for selected Gram-negative species (100/100/100%). MIC values were off-scale (resistant MIC results at ≥ 32 µg/ml) for Enterobacteriaceae and non-fermentative Gram-negative bacilli (26 strains). Overall, >99% of Sensititre MIC method results for DAL were within ± one $\log_2$ dilution of the REF MIC values. Reproducibility results showed 88.9 - 92.2% of DAL MICs were identical within and between days, and 100.0% of MIC values were within ± one $\log_2$ dilution step.

Conclusions: DAL dry-form, commercial diagnostic MIC panels have been validated for accuracy and reproducibility using 431 recent clinical isolates from four major pathogen groups within its spectrum of activity. The clinical application for this new glycopeptide appears focused against Gram-positive cocci, usually susceptible to vancomycin, teicoplanin or other glycopeptides.

INTRODUCTION

Dalbavancin (formerly BI397) is an investigational glycopeptide with an elimination half-life allowing weekly dosing with initial reports of high clinical success. The potency and spectrum of dalbavancin most closely resembles teicoplanin, however, advantages for dalbavancin include inhibition of some enterococci resistant to vancomycin (Van B phenotypes) and many coagulase-negative staphylococci (CoNS) observed to be resistant to teicoplanin.

The quality control (QC) guidelines for MIC testing of dalbavancin by the methods of the National Committee for Clinical Laboratory Standards (NCCLS) have previously been reported. Using these guidelines for reference broth microdilution methods, this study addresses the validation of dry-form, long shelf-life broth microdilution panels (Sensititre, TREK Diagnostics, Cleveland, OH, USA) to produce dalbavancin MIC results comparable to that of the NCCLS reference method. The reproducibility of the commercially produced panel MIC values for dalbavancin was also assessed.

METHODS

The protocol followed the validation recommendation published in NCCLS document M23-A2 using organism groupings within the spectrum of dalbavancin activity. The following species were tested: 100 *Streptococcus pneumoniae* (24 were non-susceptible to penicillin), 50 viridans group *Streptococcus* spp. (24 penicillin non-susceptible), 20 *S. pyogenes*, 20 *S. agalactiae*, 65 *Staphylococcus aureus* (42 oxacillin-resistant), 36 CoNS (27 oxacillin-resistant), and 101 enterococci (see Table 1 for antibiogram details). At least 100 strains from each of the four major organism groups listed in Table 1 must be tested per NCCLS guidelines (M23-A2), a criteria achieved by this experimental design.

Control vancomycin tests were performed concurrently and all MIC results were within published limits, and dalbavancin MIC results for QC strains conformed to proposed guidelines published by Anderegg et al. [2003].

Reproducibility testing utilized 10 selected Gram-positive organisms (Table 2) including four QC strains recommended by the NCCLS. Each strain was tested on three occasions daily for three consecutive days. This produced 90 replicate results to be evaluated for reproducibility within and between days of testing.

RESULTS

- Dalbavancin MIC results of testing 402 Gram-positive and 27 Gram-negative recent clinical isolates by the reference and commercial dry-form reagents are summarized in Table 1.
- Identical MIC results for dalbavancin were observed by both tests in 74.6% of tests with Gram-positive organisms.
- While MIC results for dalbavancin produced by dry-form reagents were slightly elevated compared to the reference method for staphylococci (26.7%) and enterococci (49.5%), 99.5% of all Gram-positive dalbavancin MIC results from the Sensititre panels were within ± one $\log_2$ dilution step of the reference MIC.
- Gram-negative organism tests (27 strains) had all dalbavancin MIC values at > 32 µg/ml, with complete correlation (highly resistant by both tests) between methods.
- Same day reproducibility of dry-form tests showed 83 of 90 (92.2%) results that were identical (Table 2); between day reproducibility was also excellent (80.9% identical results) with all tests (100.0%) within ± one $\log_2$ dilution step.
- QC strains (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619) had dalbavancin MIC values consistently and reproducibly at the mid-point of the proposed QC ranges.

Table 1. Validation experiments comparing the MIC results from dry-form dalbavancin panels to those MICs produced by frozen-form, reference reagents using 429 recent clinical isolates.

	Dry-form MIC variation in $\log_2$ dilution:				
Organism group (no. tested)	-2	-1	Same	+1	+2
S. pneumoniae (100)	0	5	86	9	0
Other streptococci (100)[a]	1	4	91	4	0
Staphylococci (101)[b]	0	2	72	26	1
Enterococci (101)[c]	0	0	51	50	0
Subtotal (402)	1	11	300	89	1
Gram-negative pathogens (27)[d]	0	0	27	0	0
Total (429)	1	11	327	89	1
(%)	(0.2)	(2.6)	(76.2)	(20.8)	(0.2)

a. Includes: 50 viridans group species (26 penicillin-susceptible), 20 *S. pyogenes*, 20 *S. agalactiae* and 10 strains from serogroups C, F and G.
b. Includes: 65 *S. aureus* (42 oxacillin-resistant) and 36 coagulase-negative species (27 oxacillin-resistant).
c. Includes: 61 *E. faecalis* (seven vancomycin-resistant), 30 *E. faecium* (14 vancomycin-resistant), three *E. casseliflavus*, three *E. galinarium* (one vancomycin-resistant), two *E. raffinosus* and one strain each of *E. hirae* (vancomycin-resistant) and *E. avium* (vancomycin-resistant).
d. Includes 14 species of Enterobacteriaceae or non-fermentative Gram-negative bacilli with dalbavancin MICs at > 32 µg/ml (off-scale) by both monitored methods.

Table 2. Results of a commercial panel broth microdilution MIC reproducibility experiment with dalbavancin tested against 10 organisms (includes quality control strains) with three replicates daily for three consecutive days.

	Variations in $\log_2$ dilutions for:					
	Within day replicates[a]			Between day replicates[b]		
Organism (strain no.)	-1	Same	+1	-1	Same	+1
S. mitis (15-6797A)	0	9	0	0	9	0
S. pyogenes (7-6560A)	0	8	1	0	8	1
S. pneumoniae (13-3602C)	0	8	1	0	8	1
S. pneumoniae (ATCC49619)	0	9	0	0	9	0
E. faecalis (19-6848A)	0	8	1	0	8	1
E. faecalis (ATCC 29212)	0	7	2	0	7	2
S. epidermidis (1-6777A)	0	8	1	0	8	1
S. aureus (43-507A)	0	9	0	0	9	0
S. aureus (ATCC 29213)	0	8	1	4	5	0
S. aureus (ATCC 25923)	0	9	0	0	9	0
Total (%)	0(0.0)	83(92.2)	7(7.8)	4(4.4)	80(88.9)	6(6.7)

a. Results compared to the within day consensus MIC (mode, or median if three different results were encountered).
b. Results compared to the all replicate consensus MIC value for each organism (mode from nine results).

CONCLUSIONS

- These reported results attest to the comparative accuracy and high reproducibility of dalbavancin MIC values produced by commercial (Sensititre) dry-form panels.
- As dalbavancin advances into clinical development following success in Phase II clinical trials using one or two weekly doses, its quantitative susceptibility testing can be accurately determined by MIC test products having an extended shelf-life.
- Concurrent use of QC ranges derived from NCCLS M23-A2 trial designs will further assure the quality of microbiology information to be filed with various regulatory/licensing agencies worldwide.

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