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Sulbactam-Durlobactam MIC and Disk Diffusion Quality Control Ranges Using A CLSI M23-A4 Study Design

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Abstract

Background: Sulbactam-Durlobactam (SUL-DUR) is an antibiotic designed to treat serious infections caused by *Acinetobacter baumannii*, including multidrug-resistant strains, which is currently in Phase 3 clinical testing. Durlobactam (DUR, previously ETX2514) is a diazabicyclooctane β-lactamase inhibitor with potent activity against Ambler classes A, C and D serine β-lactamases that effectively restores sulbactam (SUL) antibacterial activity against *A. baumannii*. We developed MIC and/or disk diffusion quality control (QC) ranges for SUL-DUR, SUL and DUR against two QC reference strains using multi-laboratory studies to support testing of clinical isolates.
Methods: Separate M23-A4 (Tier 2) QC studies were conducted to establish MIC and disk diffusion QC ranges for SUL-DUR and/or SUL and DUR separately against the reference strains *Escherichia coli* ATCC 25922 and *A. baumannii* NCTC 13304. Each study employed 8 reference laboratories, 3 lots of media from at least 2 manufacturers, 10 replicate tests per organism and at least 1 comparator agent per reference strain. The minimum number of testing days for each laboratory was three. For the broth MIC study, SUL-DUR was tested as doubling dilutions of SUL in the presence of a fixed concentration of 4 µg/mL DUR. Disk diffusion QC testing included 2 lots of SUL-DUR 10/10 µg disks supplied by 2 different manufacturers.
Results: Broth MIC and zone diameter QC ranges are listed in the table. A three-dilution SUL-DUR broth MIC QC range was approved by CLSI for *A. baumannii* NCTC 13304. A 6 mm zone diameter QC range was approved by CLSI for *A. baumannii* NCTC 13304 and *E. coli* ATCC 25922. *A. baumannii* NCTC 13304 is recommended for routine QC for SUL-DUR because this strain allows QC of both components of the combination.

QC organism	CLSI Approved QC Ranges			
	SUL-DUR MIC (µg/ml)	SUL MIC (µg/ml)	DUR MIC (µg/ml)	SUL-DUR 10/10 µg Disk Zone Size (mm)
A. baumannii NCTC 13304	0.5/4 - 2/4	16 - 64	32-128	24 - 30
E. coli ATCC 25922	--	16 - 64	0.12 – 0.5	26 - 32

Conclusions: The CLSI-approved broth MIC and disk diffusion QC ranges for SUL-DUR will assist clinical and reference laboratories participating in the on-going clinical trials and facilitate the regulatory review process for SUL-DUR.

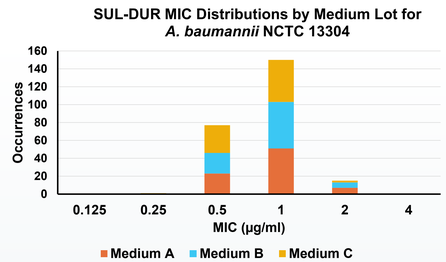
Introduction

Sulbactam-durlobactam (SUL-DUR) is an antibiotic currently in Phase 3 clinical development for the treatment of infections caused by *Acinetobacter baumannii-calcoaceticus* complex (ABC), including multidrug-resistant isolates[1]. Sulbactam (SUL) is a β-lactam inhibitor of a subset of class A β-lactamases that also has intrinsic antibacterial activity against ABC through inhibition of penicillin binding proteins PBP1 and PBP3[2]. However, degradation of SUL by a variety of β-lactamases present in most clinical ABC isolates limit its clinical use[3,4,5]. Durlobactam (DUR, formerly ETX2514) is a diazabicyclooctane (DBO) β-lactamase inhibitor with an expanded spectrum of activity compared to other DBO inhibitors, which includes coverage of a broad range of class A, C and D β-lactamases[3]. While DUR does not show clinically relevant intrinsic antibacterial activity against ABC, DUR does have activity against certain *Enterobacterales* isolates, due to inhibition of PBP2 in these organisms[3].
Clinical Laboratory Standards Institute (CLSI) M23-A4 (tier 2) quality control (QC) studies were conducted to establish broth microdilution (MIC) QC ranges for SUL-DUR, SUL and DUR as well as disk diffusion ranges for SUL-DUR.

Sulbactam-Durlobactam Broth MIC QC Ranges

Reference Strain/ Antimicrobial Agent(s)	CLSI-Approved MIC Range (µg/mL)	Percent in Range
Sulbactam-Durlobactam		
A. baumannii NCTC 13304	0.5/4 - 2/4	99.6%
E. coli ATCC 25922	No Range*	NA
Sulbactam		
A. baumannii NCTC 13304	16 - 64	98.8%
E. coli ATCC 25922	16 - 64	100%
Durlobactam		
A. baumannii NCTC 13304	32 - 128	96.7%
E. coli ATCC 25922	0.12 - 0.5	97.1%

*Because the *E. coli* DUR MIC is < 4 µg/ml, all results were off-scale, and no range could be set



- Three dilution broth MIC QC ranges were approved by CLSI for SUL-DUR, SUL alone and DUR alone[11].
- Due to the intrinsic antibacterial activity of DUR vs. *E. coli* ATCC 25922, *A. baumannii* NCTC 13304 is the only reference strain that can be used to demonstrate activity of both components of the SUL-DUR combination.
- *A. baumannii* NCTC 13304 is recommended for QC of SUL-DUR MICs

Materials and Methods

- The CLSI M23-A4 (tier 2) QC study[6] to establish broth MIC QC ranges for SUL-DUR, SUL alone and DUR alone was conducted by Clinical Microbiology Institute (Wilsonville, OR, USA).
- The CLSI M23-A4 (tier 2) QC study[6] to establish disk diffusion ranges for the SUL-DUR 10/10 µg disk was conducted by JMI Laboratories (North Liberty, IA, USA).
- For broth QC studies, the reference strains *Escherichia coli* ATCC 25922 and *A. baumannii* NCTC 13304 were used. Inoculum densities were monitored by bacterial colony counts.
- Each study used 8 reference laboratories, 3 lots of media from at least 2 manufacturers, 10 replicate tests per organism and at least one comparator agent per reference strain. The minimum number of testing days for each laboratory was three.
- For the broth MIC study, SUL-DUR was tested as doubling dilutions of SUL in the presence of a fixed concentration of 4 µg/mL DUR using CLSI methodology[7].
- For the disk diffusion study, two disk lots of SUL-DUR 10/10 µg disks were used from MAST Group and Oxoid (ThermoFisher Scientific) using CLSI methodology[8].
- For the MIC QC study, cefepime-tazobactam (fixed 8 µg/mL) and cefepime were used as controls and commercially prepared cation-adjusted Mueller-Hinton broth from Becton Dickinson (Lot #5257869), Difco (Lost #4045151) and Oxoid (Lot #1743805) were used.
- For the disk diffusion QC study, meropenem (10 µg) and ampicillin-sulbactam (10/10 µg) disks were used as controls and commercially prepared Mueller-Hinton agar from Remel (ThermoFisher Scientific) (Lot #191341), Becton Dickinson (Lot #7173648) and Hardy Diagnostics (Lot #17212) were used.
- QC Ranges for each reference strain were calculated using the Gavan statistic and RangeFinder statistical programs[9,10].

Acknowledgements

We would like to thank the following institutions for participating in the CLSI M23-A4 broth MIC QC study: Clinical Microbiology Institute (M. Traczewski), UCLA Microbiology Laboratory (J. Hindler),University of Rochester Microbiology Laboratory (D. Hardy), International Health Management Associates (D. Dressel), ThermoFisher Trek Diagnostics (C. Knapp), Laboratory Specialists Inc(L. Koeth), Tufts New England Medical Center (L. McDermott) and Micromyx (C. Pilar).
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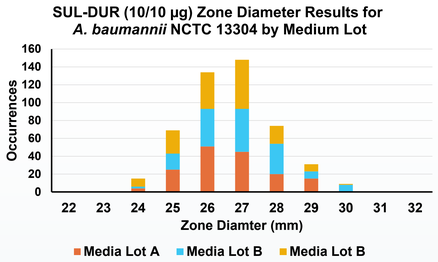
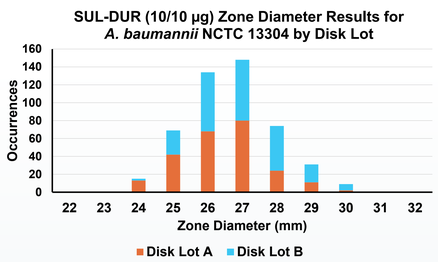
Sulbactam-Durlobactam Disk Diffusion QC Ranges

Reference Strain/ Antimicrobial Agents	CLSI-Approved Disk Diffusion Zone Diameter (µg/mL)	Percent in Range
Sulbactam-Durlobactam (10/ 10 µg)		
A. baumannii NCTC 13304*	24 – 30	100.0%
E. coli ATCC 25922	26 - 32	99.8%

*Due to the *E. coli* antibacterial activity of DUR alone, only NCTC 13304 will QC both components of the disk.

- Seven mm zone diameter QC ranges were approved by CLSI for the SUL-DUR 10/10 µg disk for *A. baumannii* NCTC 13304 and *E. coli* 25922[11].
- Due to the intrinsic antibacterial activity of DUR vs. *E. coli* ATCC 25922, *A. baumannii* NCTC 13304 is the only reference strain that can be used to demonstrate activity of both the SUL and DUR components of the disk.
- *A. baumannii* NCTC 13304 is recommended for QC of SUL-DUR disk zone sizes.





Conclusions

- **A three dilution QC range containing 99.6% of all SUL-DUR MIC values was approved by CLSI for *A. baumannii* NCTC 13304.**
- **Three dilution QC ranges containing 96.7 – 100% of all SUL or DUR MIC values were approved by CLSI for *A. baumannii* NCTC 13304 and *E. coli* ATCC 25922.**
- **7 mm QC ranges containing 99.8 – 100% of all SUL-DUR (10/10 µg) disk diffusion zone diameter values were approved by CLSI for *A. baumannii* NCTC 13304 and *E. coli* ATCC 25922.**
- ***A. baumannii* NCTC 13304 is recommended for routine QC testing of SUL-DUR MICs and disk diffusion using the SUL-DUR (10/10 µg) disk to control for both the SUL and DUR components of this β-lactam/ β-lactamase inhibitor combination.**
- **Established broth MIC and disk diffusion QC ranges for SUL-DUR will assist laboratories participating in the on-going clinical trials of SUL-DUR.**
- **If SUL-DUR is approved by the FDA, these QC ranges will also ensure that the appropriate QC standards are implemented by reference and clinical laboratories.**

References

1. NCTC03894046: https://clinicaltrials.gov/ct2/show/NCT03894046 **2.** Penwell *et al.* (2015) Antimicrob Agents Chemother. 59: 1680-1689 **3.** Durand-Reville, T. *et al.* (2017) Nature Microbiol. 2:17104. **4.** Krizova *et al.* (2013) J Antimicrob Chemother 68:2786-2791 **5.** Kuo *et al.* (2015) Front Microbiol 6:231 **6.** CLSI (2016) M23-A4 **7.** CLSI (2015) M07-A10 **8.** CLSI (2015) M02-A12. **9.** Turnidge & Bordash (2007) Antimicrob Agents Chemother. 51: 2483-2488. **10.** Gavan et al. (1981) J Clin Micro 14:67-72. **11.** CLSI (2020) M100-S30.