ESCMID 2025 | Poster #P2545

Zosurabalpin (5 µg) disk diffusion quality control ranges using Clinical and Laboratory Standards Institute M23 Tier 1 and Tier 2 criteria

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Introduction

- Antibacterial agents with activity against *Acinetobacter baumannii* and carbapenem-resistant *A. baumannii* (CRAB) isolates are urgently needed.
- Zosurabalpin (RG6006) is a novel, first-in-class, tethered macrocyclic peptide antibacterial in clinical development with specific activity against *Acinetobacter* spp. isolates, including CRAB, exerting its antimicrobial activity through inhibition of the lipopolysaccharide transporter ($LptB_2FGC$).
- In this study, we performed Clinical and Laboratory Standards Institute (CLSI) M23 (2023) Tier 1 and Tier 2 disk diffusion quality control (QC) testing using zosurabalpin (5 µg) disks against National Committee of Type Cultures (NCTC) *A. baumannii* NCTC 13304 strain to generate initial preliminary and CLSI approved quality control (QC) ranges.

Materials and Methods

- A CLSI M23 Tier 1 (single laboratory) disk diffusion QC study was conducted using 3 lots of zosurabalpin (5 µg) disks obtained from 3 different manufacturers, 1 lot of cefepime (30 µg) disks, and 4 lots of Mueller-Hinton agar (MHA) plates obtained from 4 different manufacturers, generating a total of 228 zosurabalpin (5 µg) and 76 cefepime zone diameter values.
- A minimum of 1 laboratory (Element Iowa City), 1 medium lot, 1 disk lot, and 20–30 zone diameter replicates are required for an M23 Tier 1 disk diffusion QC study.
- The CLSI M23 Tier 2 disk diffusion QC study utilized 8 participating laboratories, 2 zosurabalpin (5 µg) disk lots obtained from 2 different manufacturers, 1 cefepime (30 µg) disk lot, and 3 MHA media lots obtained from 3 different manufacturers.
- Participating laboratories in the Tier 2 study included Beth Israel Deaconess Medical Center, Element Iowa City (JMI Laboratories), Hershey Medical Center, International Health Management Associates, Microbiologics, Thermo Fisher Scientific, University of Iowa Hospitals and Clinics, and the University of Rochester Medical Center.
- Zosurabalpin (5 µg) disk diffusion susceptibility testing was conducted using 1 disk lot from 2 manufacturers on 3 different MHA media lots for 10 replicates over 3 or more days of testing (2 x 3 x 10 = 60 zone diameter values) at 8 laboratories (480 zone diameter values). A minimum of 420 zone diameter values (210 per disk lot) per QC strain from at least 7 participating laboratories are required to fulfill CLSI M23 (2023) Tier 2 criteria.
- Zosurabalpin (5 µg) zone diameter values against *A. baumannii* NCTC 13304 were read using the outer zone diameter (Figure 1).

Results

- A preliminary 7 mm disk diffusion QC range (23–29 mm) was calculated in the Tier 1 study for zosurabalpin (5 µg) disks against *A. baumannii* NCTC 13304 using the Gavan Statistic and RangeFinder statistical program that contained 99.1% (226/228) of all zosurabalpin (5 µg) zone diameter values (Table 1).
- There were ≤2 mm of variation in the mean, median, modal, and/or geometric mean zone diameter values in the Tier 1 study for zosurabalpin (5 µg) disks against *A. baumannii* NCTC 13304 between the 3 disk lots and 4 media lots tested (Table 3).
- 100.0% (76/76) of the cefepime (30 µg) zone diameter values in the Tier 1 study against *A. baumannii* NCTC 13304 were within the CLSI approved QC range of 6–16 mm (Table 1).
- A 5 mm QC range (23–27 mm) against *A. baumannii* NCTC 13304 containing 97.7% of all zosurabalpin (5 µg) zone diameter values was calculated in the Tier 2 study using the Gavan statistic (Table 2).
- In M23 Tier 2 testing, a 7 mm (22–28 mm) QC range containing 100.0% of all zosurabalpin (5 µg) zone diameter values was approved by CLSI at the January 27th, 2025, meeting in Orlando, Florida against *A. baumannii* NCTC 13304 using the RangeFinder statistical program result (Table 2 and Figures 2–3).
- There was ≤1 mm of variation in the mean, median, modal, and/or geometric mean zone diameter values in the Tier 2 study for zosurabalpin (5 µg) disks against *A. baumannii* NCTC 13304 between the 2 disk lots and 3 media lots tested (Table 4 and Figures 2–3).
- 100.0% (240/240) of the cefepime (30 µg) zone diameter values in the Tier 2 study against *A. baumannii* NCTC 13304 were within the CLSI approved QC range of 6–16 mm and provided validated results on each day of disk diffusion susceptibility testing (Table 2).

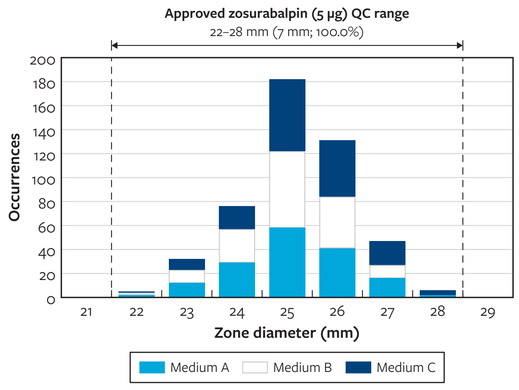
Conclusions

- The CLSI committee on Antimicrobial Susceptibility Testing approved a 7 mm zosurabalpin (5 µg) disk diffusion QC range of 22–28 mm for *A. baumannii* NCTC 13304 at the January 2025 meeting.
- The approved zosurabalpin (5 µg) disk diffusion QC range for *A. baumannii* NCTC 13304 (22–28 mm) and 3 letter abbreviation (ZAB) will be published in the 2026 CLSI M100 document.
- Establishing a CLSI disk diffusion QC range for zosurabalpin (5 µg) disks against *A. baumannii* NCTC 13304 will assist clinical laboratories and reference laboratories participating in the clinical trials.

Figure 1. Zosurabalpin (5 µg) disk outer zone diameter read for *A. baumannii* NCTC 13304

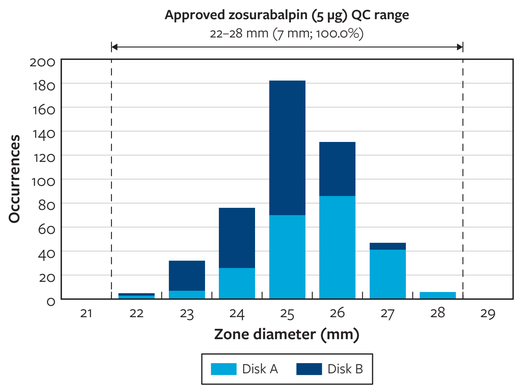
A. baumannii NCTC13304
Outer zone

Figure 2. 1 Zosurabalpin (5 µg) Tier 2 zone diameter distributions by medium lot for *Acinetobacter baumannii* NCTC 13304



A, Remel #174771; B, Carolina #2409040; C, Hardy Diagnostics #639881

Figure 3. 1 Zosurabalpin (5 µg) Tier 2 zone diameter distributions by disk lot for *Acinetobacter baumannii* NCTC 13304



Disk A, MAST Group #522631; Disk B, Liofilchem #072424077

Table 1. Preliminary zosurabalpin (5 µg) CLSI M23 Tier 1 disk diffusion quality control range

QC organism	Zosurabalpin (5 µg) Calculated zone diameter range (mm)		Cefepime (30 µg)
	Gavan statistic (mm; % in range)	RangeFinder (mm; % in range)	Current CLSI Approved QC Range (mm; % in range)
A. baumannii NCTC 13304	**23–29[a] (7; 99.1%)**	**23–29[a] (7; 99.1%)**	6–16 (11; 100.0%)

[a] Preliminary zosurabalpin (5 µg) CLSI M23 Tier 1 QC range listed in **bold**.

Table 2. CLSI approved zosurabalpin (5 µg) M23 Tier 2 disk diffusion quality control ranges

QC organism	Zosurabalpin (5 µg) Calculated zone diameter range (mm)		Cefepime (30 µg)
	Gavan statistic (mm; % in range)	RangeFinder (mm; % in range)	Current CLSI Approved QC Range (mm; % in range)
A. baumannii NCTC 13304	23–27 (5; 97.7%)	**22–28[a] (7; 100.0%)**	6–16 (11; 100.0%)

Approved zosurabalpin (5 µg) CLSI M23 Tier 2 QC range listed in **bold**.

Table 3. Zosurabalpin (5 µg) Tier 1 zone diameter values against *Acinetobacter baumannii* NCTC 13304 by disk lot and media lot

Zone diameter (mm)	Occurrence by disk lot[a]			Occurrence by media lot[b]			
	A	B	C	A	B	C	D
22							
23		4		3			1
24	4	15		12	1		6
25	24	31	5	24	13	3	20
26	25	20	16	11	18	16	16
27	19	6	28	5	16	20	12
28	4		16	2	6	10	2
29			9		3	6	
30			2			2	
31							
Total	76	76	76	57	57	57	57
Mean	25.9	25.1	27.2	25.2	26.4	27.1	25.7
Median	26	25	27	25	26	27	26
Mode	26	25	27	25	26	27	25
Geometric mean	25.9	25.1	27.2	25.1	26.4	27.1	25.6
Range (mm)	5	5	6	6	6	6	6

[a] Disk A, MAST Group #522631; Disk B, Liofilchem #072424077, Disk C, Thermo Fisher Scientific #3128
[b] A, Becton Dickinson (BD/BBL) #4169457; B, Remel #139378; C, Hardy Diagnostics #631145; D, Carolina #2407160

Table 4. Zosurabalpin (5 µg) Tier 2 zone diameter values against *Acinetobacter baumannii* NCTC 13304 by disk lot and media lot

Zone diameter (mm)	Occurrence by disk lot[a]		Occurrence by media lot[b]		
	A	B	A	B	C
21					
22	3	2	2	2	1
23	7	25	12	11	9
24	26	50	29	28	19
25	70	112	58	64	60
26	86	45	41	43	47
27	41	6	16	11	20
28	6		2		4
29					
Total	239	240	160	159	160
Mean	25.6	24.8	25.1	25.1	25.4
Median	26	25	25	25	25
Mode	26	25	25	25	25
Geometric mean	25.5	24.8	25.1	25.0	25.3
Range (mm)	7	6	7	6	7

[a] Disk A, MAST Group #522631; Disk B, Liofilchem #072424077
[b] A, Remel #139378; B, Carolina #2407160; C, Hardy Diagnostics #631145

Acknowledgments

This project was supported by F. Hoffmann-La Roche Ltd. and funded by the Department of Health and Human Services; Administration for Strategic Preparedness and Response; Biomedical Advanced Research & Development Authority (BARDA, HHSO100201600038C), including preparation of this ESCMID abstract and poster. The findings and conclusions in this poster have not been formally disseminated by the Department of Health and Human Services and should not be construed to represent any agency determination or policy.

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