

# Development of CLSI MIC and Disk Diffusion Quality Control Ranges for Cefepime/VNRX-5133

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## Abstract

**Background:** VNRX-5133 is a novel cyclic boronate Broad-Spectrum  $\beta$ -lactamase inhibitor under development by VenatoRx Pharmaceuticals, Inc. The addition of VNRX-5133 to cefepime (FEP/5133) restores the activity of cefepime against Enterobacteriaceae and *P. aeruginosa* that are resistant due to the production of certain  $\beta$ -lactamases, including ESBL, AmpC, OXA, KPC, NDM, and VIM. For control of susceptibility testing methods, this study was performed to obtain CLSI quality control ranges for both MIC broth microdilution and disk diffusion methods.

**Methods:** Disk and MIC results were obtained in accordance with CLSI M23-4 Tier 2 QC guidelines for six strains (shown in table) and included 10 replicates tested at 9 laboratories using 3 lots of media for both MIC and disk methods. MIC testing of cefepime/VNRX-5133 (fixed concentration of 4  $\mu$ g/mL) was performed by broth microdilution method along with 2 comparator agents, cefepime and cefepime/tazobactam. Disk diffusion testing was performed with cefepime/VNRX-5133 30/20  $\mu$ g disks from two manufacturers and with cefepime 30  $\mu$ g and ceftolozane/tazobactam 30/10  $\mu$ g disks. **Results:** As shown in the table, at least 98% of cefepime/VNRX-5133 results were within a 3-4 well MIC range and a 6-8 mm disk range for all strains. Comparator agent results were within established ranges for  $\geq$ 99% of results.

**Conclusions:** CLSI quality ranges have been established for six strains. CLSI has recommended that either *E. coli* NCTC 13353 or *K. pneumoniae* ATCC BAA-1705 are used as routine QC strains. The remaining strains and approved ranges are available for supplemental testing. These quality control ranges will not appear in the CLSI M100 document until the product is named; the summarized data and CLSI recommendations are available as the "June 2017 AST Meeting Minutes" on the CLSI website.

Quality Control Strain	FEP/5133 Approved CLSI Range (% within Range)	
	MIC ( $\mu$ g/mL)	Disk (mm)
<i>E. coli</i> ATCC 25922	0.03/4 - 0.12/4 (100%)	31-37 (99.0%)
<i>E. coli</i> ATCC 35218 (TEM-1)	0.016/4-0.06/4 (100%)	31-37 (98.7%)
<i>E. coli</i> NCTC 13353 (CTX-M-15)	0.12/4-1/4 (99.6%)	24-30 (99.8%)
<i>K. pneumoniae</i> ATCC 700603 (SHV-18)	0.12/4-0.5/4 (99.3%)	24-31 (99.4%)
<i>K. pneumoniae</i> ATCC BAA-1705 (KPC)	0.12/4-0.5/4 (98.9%)	22-27 (99.0%)
<i>P. aeruginosa</i> ATCC 27853	0.5/4-4/4 (100%)	25-31 (99.4%)

## Introduction

- VNRX-5133 is a new injectable cyclic boronate broad-spectrum  $\beta$ -lactamase inhibitor which is in clinical development for the treatment of Gram negative infections. The addition of VNRX-5133 to cefepime (FEP/5133) restores the activity of cefepime against Enterobacteriaceae and *P. aeruginosa* that are resistant due to the production of serine  $\beta$ -lactamases and the emerging metallo-beta-lactamases, including ESBL, AmpC, OXA, KPC, NDM, and VIM.
- This multi-laboratory QC study of cefepime/VNRX-5133 was conducted according to CLSI M23-A4 Tier 2 QC testing requirements for the development of MIC and disk diffusion quality control ranges.

## References:

- CLSI. Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters- 4th ed. CLSI guideline M23-A4 Wayne, PA: Clinical and Laboratory Standards Institute; 2015
- Clinical and Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 10<sup>th</sup> ed. Approved standard, CLSI M7-10, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2017 Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard – 27<sup>th</sup> Edition. CLSI document M100-27 Wayne, PA

## Methods

Study Strains	Testing Sites
<i>Escherichia coli</i> ATCC 25922	Clinical Microbiology Institute, Wilsonville, OR
<i>Escherichia coli</i> ATCC 35218 (TEM-1 producer)	International Health Management Association, Schaumburg, IL
<i>Escherichia coli</i> NCTC 13353 (CTX-M-15 producer)	JMI Laboratories, North Liberty, IA
<i>Klebsiella pneumoniae</i> ATCC 700603 (SHV-18 ESBL producer)	Laboratory Specialists, Inc., Westlake, OH
<i>Klebsiella pneumoniae</i> ATCC BAA-1705 (KPC producer)	Micromyx, Kalamazoo, MI
<i>Pseudomonas aeruginosa</i> ATCC 27853	Thermo Fisher Scientific, Oakwood Village, OH
	University Hospital Cleveland Medical Ctr., Cleveland, OH
	University of Rochester Medical Center, Rochester, NY

MIC testing according to CLSI M7-A10 with CAMHB from 3 Manufacturers:	Disk Diffusion testing with Cefepime/VNRX5133 30/20 $\mu$ g disks with 3 MHB from 3 Manufacturers and disks from 2 Manufacturers:	
	Mueller Hinton Agar	Cefepime/VNRX-5133 30/20 $\mu$ g Disks
Difco, Becton Dickinson, Sparks, MD	BBL, Becton Dickinson, Sparks, MD	Oxoid, Basingstoke, UK
Becton Dickinson, Sparks, MD	Hardy Diagnostics, Santa Maria, CA	Mast, Merseyside, UK
Oxoid, Remel, Lenexa, KS	Remel, Lenexa, KS	

Comparator agents (MIC)	Comparator agents (disk)
Cefepime	Cefepime (30 $\mu$ g, Mast, Hardy Diagnostics)
Cefepime/tazobactam (at fixed concentration of 8 $\mu$ g/mL)	Ceftolozane/tazobactam (30/10 $\mu$ g, Mast, Hardy Diagnostics)

## Results

All Cefepime/VNRX-5133 MIC and disk results are shown in Figure 1.

- Comparator agent results:** for those agents and study isolates with existing CLSI QC ranges, results were within the established ranges for  $>$ 98.9% of results, with exception of one site (see site variation).
- Site variation:** Disk results from one site, with exception of *P. aeruginosa* ATCC 27853 data, were not utilized in the final analysis as a result of outlier QC results for cefepime. There was some minor site variation noted for both MIC and disk results with exception of MIC results for *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC BAA-1705, which were very reproducible across all sites.
- MIC media variation:** There was a slight tendency for lower MIC results with Difco broth for the 3 *E. coli* strains.
- Disk media variation:** There was a slight tendency for smaller zones of inhibition with BBL agar for all strains, with exception of *K. pneumoniae* ATCC BAA-1705.
- Disk variation:** There was good correlation between the two disks; no significant variation was noted.
- MIC and disk reading issues:** There were no reports of difficult to read MIC endpoints or disk zones.

## Conclusions

The following Cefepime/VNRX-5133 QC ranges were presented and approved by CLSI June 2017:

Quality Control Strain	CLSI Approved Range (% within Range)	
	MIC ( $\mu$ g/mL)	Disk (mm)
<i>E. coli</i> ATCC 25922	0.03/4 - 0.12/4 (100%)	31-37 (99.0%)
<i>E. coli</i> ATCC 35218	0.016/4-0.06/4 (100%)	31-37 (98.7%)
<i>E. coli</i> NCTC 13353*	0.12/4-1/4 (99.6%)	24-30 (99.8%)
<i>K. pneumoniae</i> ATCC 700603	0.12/4-0.5/4 (99.3%)	24-31 (99.4%)
<i>K. pneumoniae</i> ATCC BAA-1705*	0.12/4-0.5/4 (98.9%)	22-27 (99.0%)
<i>P. aeruginosa</i> ATCC 27853	0.5/4-4/4 (100%)	25-31 (99.4%)

\*EC 13353 or KP BAA-1705 are recommended routine QC strains for testing of Cefepime/VNRX-5133 and Cefepime

Figure 1. Cefepime/VNRX-5133 MIC and disk diffusion by testing site, media and disk manufacturer for 6 QC strains

