

Meropenem-Vaborbactam (Carbavance™) MIC and Zone Diameter Quality Control Ranges

Using a CLSI M23-A4 Multi-Laboratory Study Design

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Amended Abstract

Background: Vaborbactam (formerly RPX7009) is a cyclic boronic acid β -lactamase inhibitor with activity against class A and C β -lactamases that was specifically optimized for increased potency against *Klebsiella pneumoniae* carbapenemase (KPC). A phase 3 clinical trial of meropenem-vaborbactam (MEV) in adult patients (>18 years) with cUTI has recently completed; the efficacy and safety of the combination is currently being investigated in patients with serious infections due to carbapenem-resistant *Enterobacteriaceae*. We developed MIC and/or disk diffusion quality control (QC) ranges for MEV and meropenem against 8 QC reference strains to support ongoing testing of clinical trial isolates in reference laboratories.

Methods: Separate M23-A4 (Tier 2) QC studies were conducted to establish MIC and disk diffusion QC ranges for MEV and/or meropenem against 8 ATCC QC reference strains. Each study employed ≥ 7 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. Disk diffusion QC testing included 2 lots of MEV disks supplied by 2 manufacturers.

Results: Broth microdilution and zone diameter QC ranges for MEV are listed in the table. Three-dilution MEV broth microdilution QC ranges were approved by CLSI for *Staphylococcus aureus* ATCC 29213, *K. pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC BAA-2814 (KPC-3, TEM-1, SHV-11), whereas 4-dilution MEV QC ranges were approved for *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853. A 7 mm zone diameter QC range was the most required for MEV 20/10 μ g disks against the 6 QC strains tested. The previously approved broth microdilution QC range of 0.015/8-0.06/8 μ g/mL for *K. pneumoniae* ATCC BAA-1705 (KPC-2) was expanded to 4 dilutions based on the bimodal distribution of data.

Meropenem-vaborbactam (fixed 8 μ g/mL) approved CLSI QC ranges		
QC organism (ATCC no.)	MIC (μ g/mL)	20/10 μ g disk zone size (mm)
<i>Staphylococcus aureus</i> ATCC 29213	0.03/8 – 0.12/8	→
<i>S. aureus</i> ATCC 25923	→ ^a	32 – 38
<i>Escherichia coli</i> ATCC 25922	0.008/8 – 0.06/8	31 – 37
<i>E. coli</i> ATCC 35218	0.008/8 – 0.06/8	→
<i>Klebsiella pneumoniae</i> ATCC 700603	0.015/8 – 0.06/8	29 – 35
<i>K. pneumoniae</i> ATCC BAA-1705	0.015/8 – 0.06/8 ^b	21 – 27
<i>K. pneumoniae</i> ATCC BAA-2814	0.008/8 – 0.06/8	16 – 20
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.12/8 – 1/8	29 – 35

^a Not tested
^b Previously approved QC range

Conclusions: The recently approved broth microdilution and disk diffusion QC ranges for MEV should assist clinical and reference laboratories participating in clinical trials and facilitate the regulatory review process for meropenem-vaborbactam.

Introduction

- Meropenem-vaborbactam is a novel antibacterial combination consisting of a cyclic boronic acid β -lactamase inhibitor with activity against class A and C β -lactamases (including KPC) and a carbapenem
- A phase 3 clinical trial of meropenem-vaborbactam in adult patients (>18 years) with cUTI has recently completed; the efficacy and safety of meropenem-vaborbactam is currently being investigated in patients with serious infections due to carbapenem-resistant *Enterobacteriaceae*
- Clinical and Laboratory Standards Institute (CLSI) M23-style (Tier 2) quality control studies were conducted to establish meropenem-vaborbactam broth microdilution (vaborbactam at fixed 8 μ g/mL) and zone diameter (20/10- μ g disks) quality control (QC) ranges against CLSI QC reference strains
- Meropenem-vaborbactam broth microdilution and zone diameter QC ranges will assist both clinical and reference laboratories in generating reliable/accurate susceptibility testing results during clinical trials and in clinical microbiology practice

Materials and Methods

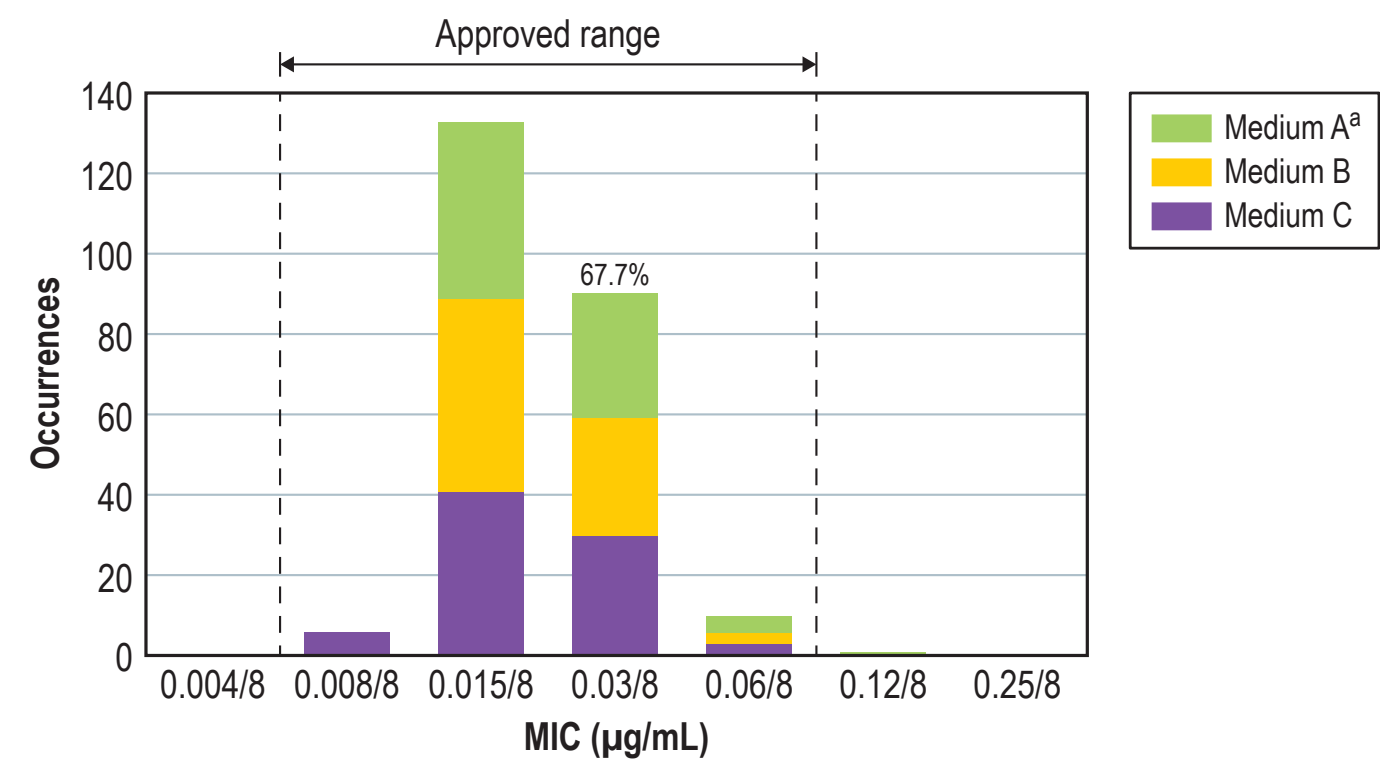
- A listing of investigators and institutions participating in CLSI M23-A4 (2016) broth microdilution (BMD) and/or disk diffusion (DD) QC studies for meropenem-vaborbactam is detailed in Table 1
- BMD and DD M23 studies utilized a minimum of seven participating laboratories (Table 1), three lots of Mueller-Hinton medium obtained from at least two different manufacturers, and ≥ 10 replicate tests per QC strain
- Mueller-Hinton medium for BMD testing was obtained from Difco (Detroit, Michigan), Becton Dickinson (BD; Sparks, Maryland), Remel (Thermo Fisher Scientific; Lenexa, Kansas), and/or Oxoid (Hampshire, United Kingdom)
- Mueller-Hinton agar plates for DD testing were obtained from Hardy Diagnostics (Santa Maria, California), Remel (Thermo Fisher Scientific; Lenexa, Kansas), and BBL (BD; Sparks, Maryland)
- Frozen-form BMD susceptibility panels were prepared in a certified GMP facility (Trek Diagnostic Systems/Thermo Fisher Scientific, Oakwood Village, Ohio) using meropenem and vaborbactam powders provided by The Medicines Company
- DD testing employed two lots of meropenem-vaborbactam (20/10 μ g) disks obtained from two different manufacturers (Bio-Rad Laboratories; Hercules, California, and Mast Group; Bootle, Merseyside, United Kingdom) and meropenem (10 μ g) control disks obtained from BD (Sparks, Maryland)
- Two KPC-producing isolates of *K. pneumoniae* (ATCC BAA-1705 and ATCC BAA-2814) were introduced as QC reference strains specifically to address the activity of meropenem and vaborbactam; these two reference strains allow for QC of meropenem-vaborbactam across a range of susceptibility concentrations

Results

- Applying CLSI M23 (Tier 2) analysis criteria to meropenem-vaborbactam, $\geq 95\%$ of meropenem-vaborbactam MIC and DD zone diameter results from the participating laboratories (Table 2) were within the approved QC ranges recently published in the CLSI M100-S27 document for each of the QC reference strains tested (Table 2 and Figures 1-4)
- Three dilution BMD QC ranges were approved for meropenem-vaborbactam against *S. aureus* ATCC 29213, *K. pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC BAA-2814 (Table 2 and Figure 2), whereas four dilution QC ranges were approved for *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC BAA-1705, and *P. aeruginosa* ATCC 27853 based on the bimodal distribution of data (Table 2 and Figure 1)
- Of the recently approved broth microdilution QC ranges for meropenem-vaborbactam, only *K. pneumoniae* ATCC BAA-1705 (KPC-2) and *K. pneumoniae* ATCC BAA-2814 (KPC-3, TEM-1, SHV-11) can adequately address both the meropenem and vaborbactam components of the antimicrobial combination and should be tested routinely when evaluating the activity of this combination
- Seven millimeter DD QC ranges were approved for meropenem-vaborbactam (20/10 μ g) against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and ATCC BAA-1705, and *P. aeruginosa* ATCC 27853, whereas a 5 mm DD QC range was approved for meropenem-vaborbactam disks against *K. pneumoniae* ATCC BAA-2814 (Table 2 and Figures 3-4)
- For the QC reference strains tested, 99.0% of meropenem MIC results and 99.8% of meropenem (10 μ g) disk diffusion results were within CLSI published QC ranges, providing validated internal controls for these studies
- Slightly larger mean zone diameters (0.1 – 1.0 mm) were observed with meropenem-vaborbactam (20/10 μ g) disks obtained from Bio-Rad than those obtained from Mast Group
- Colony counts were performed on each of the QC reference strains tested (Table 3) and results were within acceptable inoculum targets

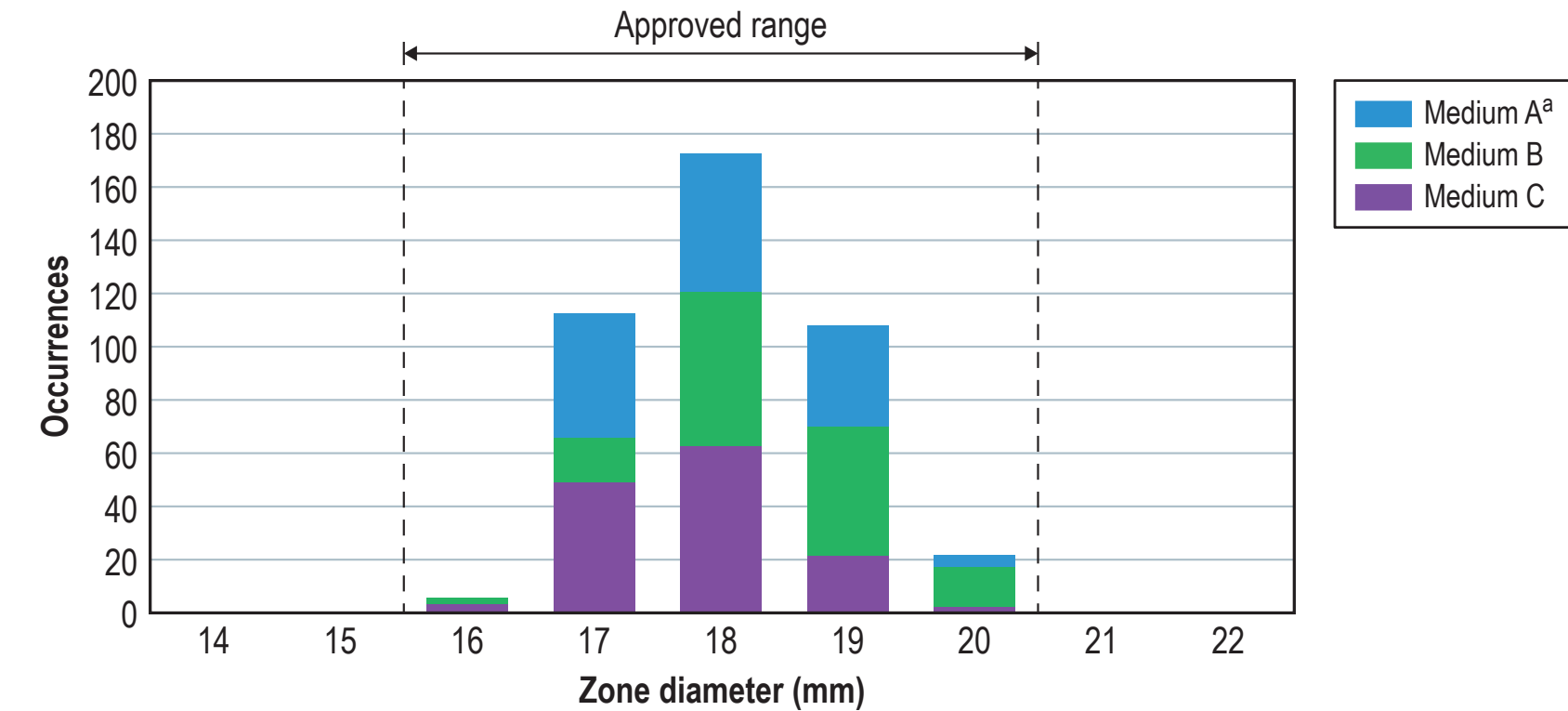
Results

Figure 1 Meropenem-vaborbactam (fixed 8 μ g/mL) MIC distributions by medium lot for *K. pneumoniae* ATCC BAA-1705 (KPC-2)



^a Medium A = Beckton Dickinson; Medium B = Difco; Medium C = Oxoid

Figure 4 Meropenem-vaborbactam (20/10 μ g) zone diameter distributions by medium lot for *K. pneumoniae* ATCC BAA-2814 (KPC-3, TEM-1, SHV-11)



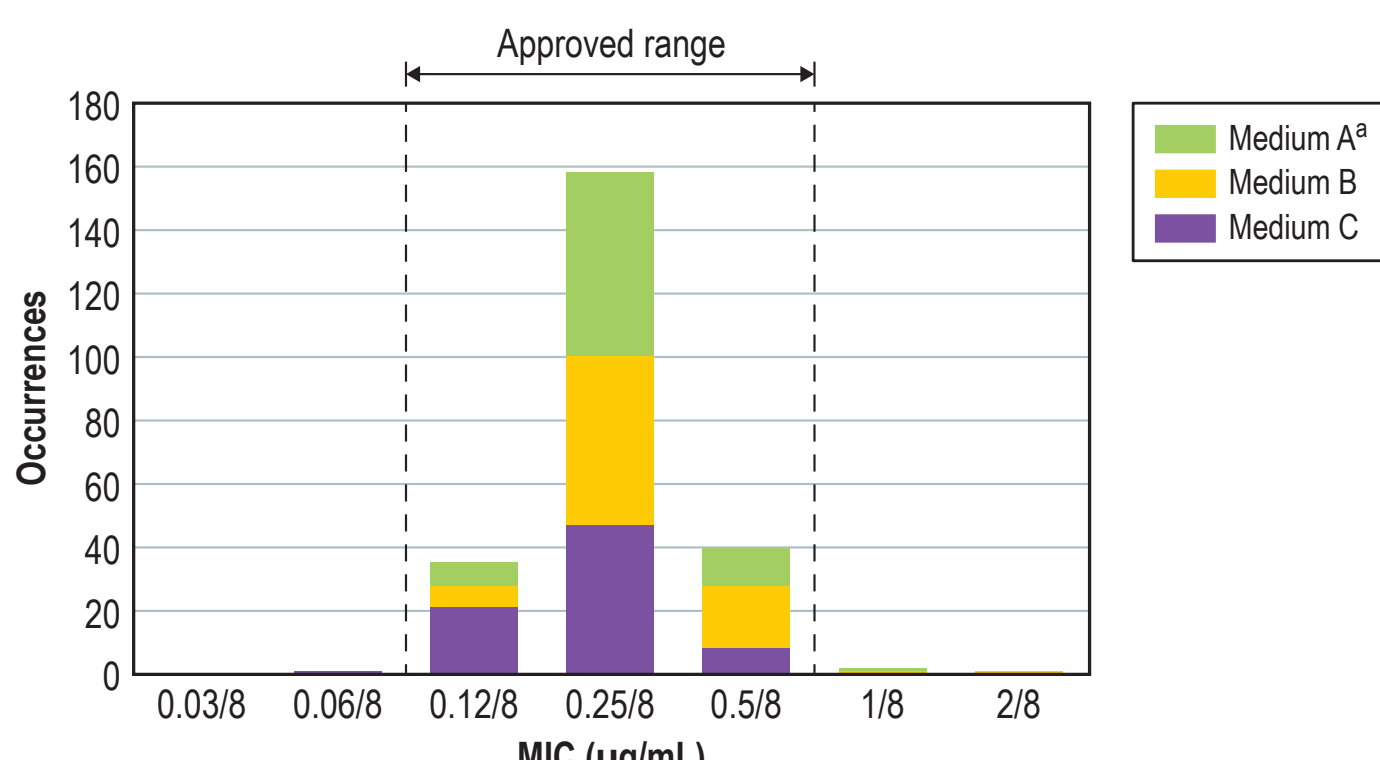
^a Medium A = Hardy Diagnostics; Medium B = Remel; Medium C = BBL

Table 2 CLSI approved BMD and DD zone diameter ranges for meropenem-vaborbactam and meropenem

CLSI QC reference strain	MIC range (μ g/mL)		Zone diameter range (mm)	
	Meropenem-vaborbactam	Meropenem	Meropenem-vaborbactam (20/10 μ g)	Meropenem (10 μ g)
<i>Staphylococcus aureus</i> ATCC 29213	0.03/8 – 0.12/8	0.03 – 0.12	N/A ^a	N/A
<i>S. aureus</i> ATCC 25923	N/A	N/A	32 – 38 (7 mm)	29 – 37 (9 mm)
<i>Escherichia coli</i> ATCC 25922	0.008/8 – 0.06/8	0.008 – 0.06	31 – 37 (7 mm)	28 – 34 (7 mm)
<i>E. coli</i> ATCC 35218	0.008/8 – 0.06/8	0.015 – 0.06 ^b	→ ^c	→ ^c
<i>Klebsiella pneumoniae</i> ATCC 700603	0.015/8 – 0.06/8	0.015 – 0.06 ^b	29 – 35 (7 mm)	28 – 34 ^d
<i>K. pneumoniae</i> ATCC BAA-1705	0.008/8 – 0.06/8	8 – 64	21 – 27 (7 mm)	11 – 18 ^d
<i>K. pneumoniae</i> ATCC BAA-2814	0.12/8 – 0.5/8	32 – 256	16 – 20 (5 mm)	6 ^d
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.12/8 – 1/8	0.12 – 1	29 – 35 (7 mm)	27 – 33 (7 mm)

^a N/A, not applicable
^b MIC ranges based on a single lot of media tested
^c →, not tested
^d Zone diameter based on a single disk lot tested

Figure 2 Meropenem-vaborbactam (fixed 8 μ g/mL) MIC distributions by medium lot for *K. pneumoniae* ATCC BAA-2814 (KPC-3, TEM-1, SHV-11)



^a Medium A = Beckton Dickinson; Medium B = Difco; Medium C = Oxoid

Table 1 Investigators and laboratories participating in meropenem-vaborbactam M23 BMD and/or DD quality control testing

Investigator	Laboratory and location	M23 study participation ^a
G. Kallstrom, Ph.D.	Summa Health Systems, Akron, OH	BMD, DD
M. Castanheira, Ph.D.	JMI Laboratories, North Liberty, IA	BMD, DD
C. Knapp, M.S.	Thermo Fisher Scientific, Cleveland, OH	BMD, DD
G. Procop, Ph.D.	Cleveland Clinic, Cleveland, OH	BMD
S. Swamy, B.S.	University of Washington Medical Center, Seattle, WA	BMD
E. Munson, Ph.D.	Wheaton Franciscan Laboratory, Wauwatosa, WI	BMD, DD
S. Riedel, M.D., Ph.D.	Johns Hopkins Bayview Medical Center, Baltimore, MD	BMD
R. Rennie, Ph.D.	University of Alberta Hospitals, Edmonton, Alberta, Canada	BMD, DD
D. Hardy, Ph.D.	University of Rochester Medical Center, Rochester, NY	BMD, DD
T. Fritzsche, M.D., Ph.D.	Marshfield Laboratories, Marshfield, WI	BMD
C. Pillar, Ph.D.	Micromyx Inc., Kalamazoo, MI	BMD
G. Denys, Ph.D.	Indiana University Health, Methodist Hospital, Indianapolis, IN	BMD, DD

^a BMD, broth microdilution; DD, disk diffusion

Table 3 Colony counts for the quality control reference strains used in BMD testing

CLSI QC reference strain	Range ^a (CFU/mL)	Average ^b (CFU/mL)
<i>Staphylococcus aureus</i> ATCC 29213	0.5 x 10 ⁵ – 7.9 x 10 ⁵	3.5 x 10 ⁵
<i>Escherichia coli</i> ATCC 25922	1.1 x 10 ⁵ – 6.2 x 10 ⁵	3.0 x 10 ⁵
<i>E. coli</i> ATCC 35218	0.3 x 10 ⁵ – 7.2 x 10 ⁵	3.1 x 10 ⁵
<i>Klebsiella pneumoniae</i> ATCC 700603	1.0 x 10 ⁵ – 7.6 x 10 ⁵	2.9 x 10 ⁵
<i>K. pneumoniae</i> ATCC BAA-1705	2.1 x 10 ⁵ – 7.7 x 10 ⁵	3.3 x 10 ⁵
<i>K. pneumoniae</i> ATCC BAA-2814	1.0 x 10 ⁵ – 7.6 x 10 ⁵	3.5 x 10 ⁵
<i>Pseudomonas aeruginosa</i> ATCC 27853	1.3 x 10 ⁵ – 8.0 x 10 ⁵	4.5 x 10 ⁵

^a Range of all participating laboratories
^b Average of all participating laboratories

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Conclusions

- Meropenem-vaborbactam (fixed 8 μ g/mL) broth microdilution susceptibility testing demonstrated acceptable inter- and intra-laboratory reproducibility with the following CLSI QC reference strains: *S. aureus* ATCC 29213; *E. coli* ATCC 25922 and ATCC 35218; *K. pneumoniae* ATCC 700603, BAA-1705, and BAA-2814; and *P. aeruginosa* ATCC 27853
- K. pneumoniae* ATCC BAA-1705 (KPC-2) and/or *K. pneumoniae* ATCC BAA-2814 (KPC-3, TEM-1, SHV-11) should be utilized as QC reference strains when evaluating the activity of meropenem-vaborbactam combinations as they provide QC on both the meropenem and vaborbactam components of the antimicrobial combination
- Meropenem-vaborbactam (20/10 μ g) disk diffusion susceptibility testing demonstrated acceptable inter- and intra-laboratory reproducibility with the following CLSI QC reference strains: *S. aureus* ATCC 25923; *E. coli* ATCC 25922; *K. pneumoniae* ATCC 700603, BAA-1705, and BAA-2814; and *P. aeruginosa* ATCC 27853
- The CLSI subcommittee on Antimicrobial Susceptibility Testing approved meropenem-vaborbactam broth microdilution and disk diffusion QC ranges against reference strains at the January 2015, June 2016, and January 2017 meetings. These approved meropenem-vaborbactam QC ranges were recently published in Tables 4A and 5A of the CLSI M100-S27 document
- The approved broth microdilution and disk diffusion QC ranges for meropenem-vaborbactam will assist both clinical and reference laboratories and facilitate the regulatory review process for meropenem-vaborbactam

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