

Biapenem and Biapenem/RPX7009 MIC Quality Control Ranges Using CLSI Multi-laboratory M23-A3 Study Design

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Abstract

OBJECTIVES: To conduct a study to establish MIC quality control (QC) ranges for biapenem (BPM) and BPM in combination with RPX7009, a β -lactamase inhibitor, using the broth microdilution (BMD) method. This broad-spectrum combination has activity against key Gram-negative pathogens including *E. coli*, *P. aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp. and those organisms that produce serine beta-lactamases like KPC.

METHODS: An eight laboratory study design was compliant with CLSI M23-A3 guidelines. Six QC strains were tested (*S. aureus* ATCC 29213 [SA], *E. coli* ATCC 25922 [EC1], *E. coli* ATCC 35218 [EC2], *K. pneumoniae* ATCC 700603 [KPI], *K. pneumoniae* BAA 1705 [KP2], and *P. aeruginosa* ATCC 27853 [PA]) using three media lots (three manufacturers) of cation-adjusted Mueller-Hinton broth (MHB). Ten replicate tests were performed for each QC organism generating 720 BMD values/strain (4,320 total). Meropenem, piperacillin/tazobactam, and amoxicillin/clavulanic acid were used as controls.

RESULTS: The table lists the proposed MIC QC ranges for BPM and BPM/RPX7009. An alternative range suggested by the Range Finder statistical program was a four log₂ dilution range for KPI (0.03 – 0.25 mg/L). A four log₂ dilution range was required for KP2 (KPC-producing strain) with BPM/RPX7009 at both a fixed 4 and 8 mg/L concentration due to a “shoulder” MIC at 0.03 mg/L, which had 68.2% and 85.8%, respectively, of the MIC values compared to the modal occurrences at 0.06 mg/L. A range was established for BPM alone with KP2 at >1 mg/L to be added to the appropriate CLSI table footnote and compared to the significantly lower MIC results for the combination. There were no differences in proposed QC ranges between RPX7009 fixed 4 mg/L concentration or RPX7009 fixed 8 mg/L for all six QC organisms. No significant differences were noted among MHB media lots for BPM or BPM/RPX7009. Nearly all (714/720; 99.3%) control agent MIC results were within CLSI published ranges. Meropenem MIC results for the KP2 strain were all greater than 0.5 mg/L.

CONCLUSIONS: Proposed MIC QC ranges for BPM and BPM/RPX7009 should guide clinical or reference laboratories participating in the testing of clinical trial isolates and facilitate the regulatory review process for this investigational antimicrobial combination.

QC organism (ATCC no.)	Proposed QC ranges for BMD tests (MIC in mg/L; % in proposed range)		
	Biapenem	Biapenem/RPX7009 fixed 4 mg/L	Biapenem/RPX7009 fixed 8 mg/L
SA 29213	0.3 – 0.12 (100.0)	0.03 – 0.12 (100.0)	0.03 – 0.12 (100.0)
EC1 25922	0.03 – 0.12 (100.0)	0.03 – 0.12 (100.0)	0.03 – 0.12 (99.6)
EC2 35218	0.03 – 0.12 (100.0)	0.03 – 0.12 (100.0)	0.03 – 0.12 (100.0)
KPI 700603	0.03 – 0.12 (95.0)	0.03 – 0.12 (97.5)	0.03 – 0.12 (99.2)
KP2 BAA1705	>1 (100.0)	0.015 – 0.12 (97.5)	0.015 – 0.12 (99.2)
PA 27853	0.5 – 2 (100.0)	0.5 – 2 (100.0)	0.5 – 2 (99.6)

Introduction

A broad-spectrum beta-lactamase inhibitor, RPX7009, is combined with a carbapenem (biapenem) at two fixed concentrations. This novel drug combination will help address the emerging problem of class A carbapenemases, such as KPC and related enzymes among important, prevalent Gram-negative pathogens.

This broth microdilution quality control (QC) study of biapenem and biapenem combined with RPX7009 was performed following the Clinical and Laboratory Standards Institute (CLSI) M23-A3 (2008) guideline document using eight participant laboratories, different manufacturers of media and three antimicrobial control agents. The results are presented as proposed QC ranges in mg/L concentrations for six American Type Culture Collection (ATCC) strains: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 and ATCC BAA1705 as well as *Pseudomonas aeruginosa* ATCC 27853.

Materials and Methods

A total of eight laboratories were recruited to provide data for this QC investigation. Broth microdilution panels included three cation-adjusted Mueller-Hinton (MH) broth media lots produced by Difco Laboratories (Detroit, Michigan, USA), Becton Dickinson (BD; Sparks, Maryland, USA), and Oxoid (Hampshire, United Kingdom [UK]). Biapenem and RPX7009 were provided by Rempex Pharmaceuticals Inc (San Diego, California, USA); meropenem, piperacillin/tazobactam, and amoxicillin/clavulanic acid were acquired from Sigma-Aldrich (St. Louis, Missouri, USA). Panels were prepared by a certified GMP source (ThermoFisher Scientific, Cleveland, Ohio, USA). Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates.

Ten replicates of each ATCC strain produced 1,440 MIC values for biapenem, biapenem/RPX7009 at fixed 4 mg/L and biapenem/RPX7009 at fixed 8 mg/L. The *K. pneumoniae* ATCC BAA1705 strain (carbapenemase [*bla*_{KPC}] producing) was included in the study to control for the beta-lactamase inhibitor activity. This strain is used as a positive control for the Modified Hodge Test to detect carbapenemases in clinical isolates. All QC organisms were incubated at 16–20 hours at 35°C in ambient air according to CLSI document M07-A9 (2012). All sites were instructed to read the MIC endpoint at 100% or complete inhibition of growth.

Results

- Colony counts were performed on the panels with the average colony counts for the participating centers ranging from 1.7 X 10⁵ to 5.2 X 10⁵ CFU/ml for *S. aureus* ATCC 29213; 2.0 X 10⁵ to 5.0 X 10⁵ CFU/ml for *E. coli* ATCC 25922; 1.6 X 10⁵ to 5.1 X 10⁵ CFU/ml for *E. coli* ATCC 35218; 2.6 X 10⁵ to 5.6 X 10⁵ CFU/ml for *K. pneumoniae* ATCC 700603; 1.8 X 10⁵ to 5.7 X 10⁵ CFU/ml for *K. pneumoniae* ATCC BAA1705 and 1.8 X 10⁵ to 4.6 X 10⁵ CFU/ml for *P. aeruginosa* ATCC 27853; all acceptable.
- The biapenem and biapenem/RPX7009 with inhibitor at fixed 4 and 8 mg/L MIC results for *S. aureus* ATCC 29213 are all (100.0%) within the proposed limits of 0.03 – 0.12 mg/L (mode ± one log₂ dilution step).
- The proposed QC ranges were the same for both *E. coli* strains (ATCC 25922 and 35218) for all three investigational agents. The range of 0.03 – 0.12 mg/L included all *E. coli* results.
- A range of 0.03 – 0.12 mg/L was also established for *K. pneumoniae* ATCC 700603 with 95.0% of results included in the proposed range for biapenem, 97.5% for biapenem/RPX7009 at fixed 4 mg/L and 99.2% for biapenem/RPX7009 at fixed 8 mg/L.

TABLE 1. Proposed Quality Control Ranges for Broth Microdilution Testing for Biapenem, Biapenem/RPX7009 at Fixed 4 and 8 mg/L.

QC organism	Antimicrobial	MIC range (mg/L)	% in proposed range
<i>S. aureus</i> ATCC 29213	Biapenem	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 4 mg/L	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 8 mg/L	0.03 – 0.12	100.0
<i>E. coli</i> ATCC 25922	Biapenem	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 4 mg/L	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 8 mg/L	0.03 – 0.12	99.6
<i>E. coli</i> ATCC 35218	Biapenem	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 4 mg/L	0.03 – 0.12	100.0
	Biapenem/RPX7009 fixed 8 mg/L	0.03 – 0.12	100.0
<i>K. pneumoniae</i> ATCC 700603	Biapenem	0.03 – 0.12	95.0
	Biapenem/RPX7009 fixed 4 mg/L	0.03 – 0.12	97.5
	Biapenem/RPX7009 fixed 8 mg/L	0.03 – 0.12	99.2
<i>K. pneumoniae</i> ATCC BAA1705	Biapenem	>1	100.0
	Biapenem/RPX7009 fixed 4 mg/L	0.015 – 0.12	97.5
	Biapenem/RPX7009 fixed 8 mg/L	0.015 – 0.12	99.2
<i>P. aeruginosa</i> ATCC 27853	Biapenem	0.5 – 2	100.0
	Biapenem/RPX7009 fixed 4 mg/L	0.5 – 2	100.0
	Biapenem/RPX7009 fixed 8 mg/L	0.5 – 2	99.6

- Figures 1 and 2 show *K. pneumoniae* ATCC BAA1705 (KPC-producer) MIC results with a proposed range of 0.015 – 0.12 mg/L for both inhibitor concentrations. This proposed four log₂ dilution range included 97.4% of reported results for biapenem/RPX7009 at fixed 4 mg/L and 99.2% for biapenem/RPX7009 at fixed 8 mg/L. Figures 1 and 2 illustrate the prominent “shoulder” phenomenon at 0.03 mg/L.
- P. aeruginosa* ATCC 27853 has the same proposed range of 0.5 – 2 mg/L for biapenem alone or with all concentrations of RPX7009 tested. Only the biapenem/RPX7009 at fixed 8 mg/L had one MIC value outside of the proposed range.
- The internal control results for meropenem, piperacillin/tazobactam, and amoxicillin/clavulanic acid were predominately within the CLSI published range (714 of 720 results; 99.3%) providing a validated internal control for this investigation.
- There was no significant difference in performance among the tested lots of Mueller-Hinton broth.

FIGURE 1. Biapenem/RPX7009 Fixed 4 mg/L MIC Distributions for *K. pneumoniae* ATCC BAA1705.

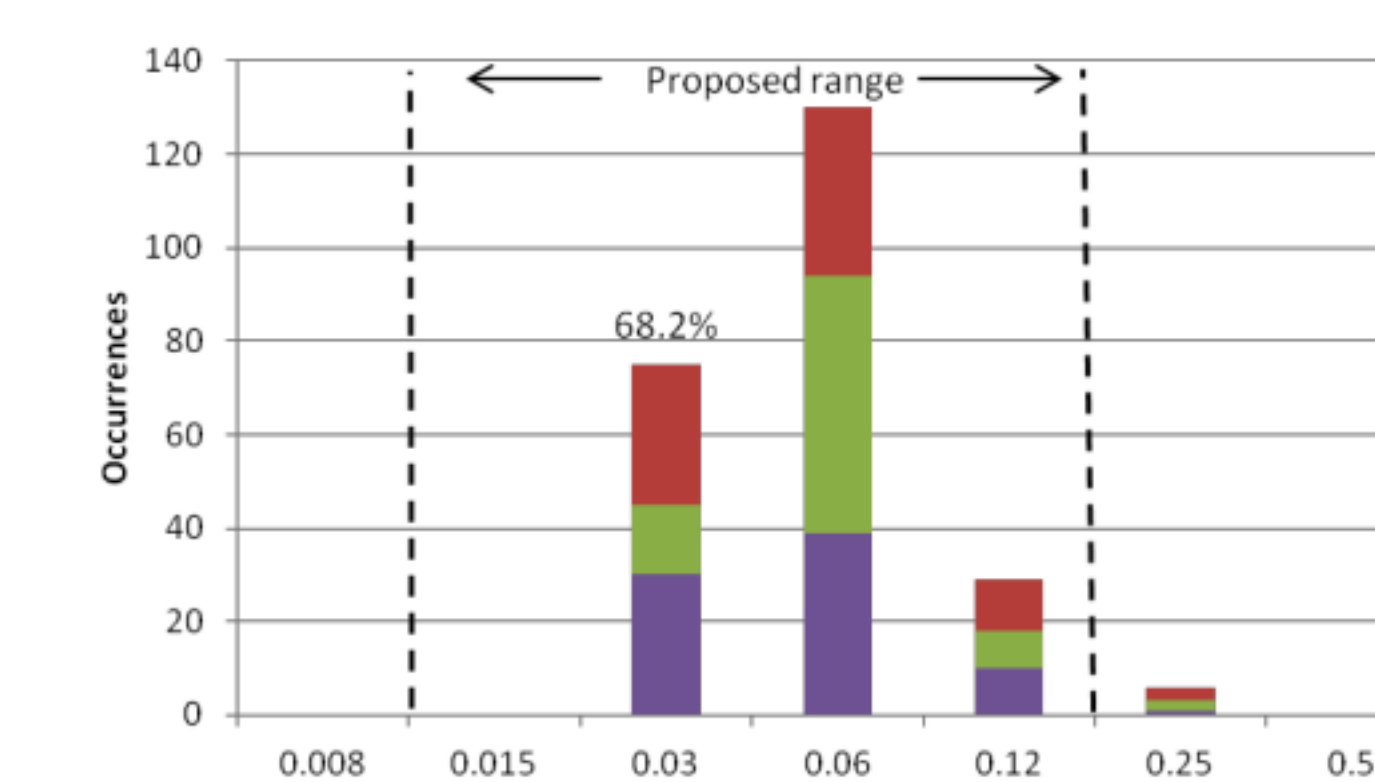
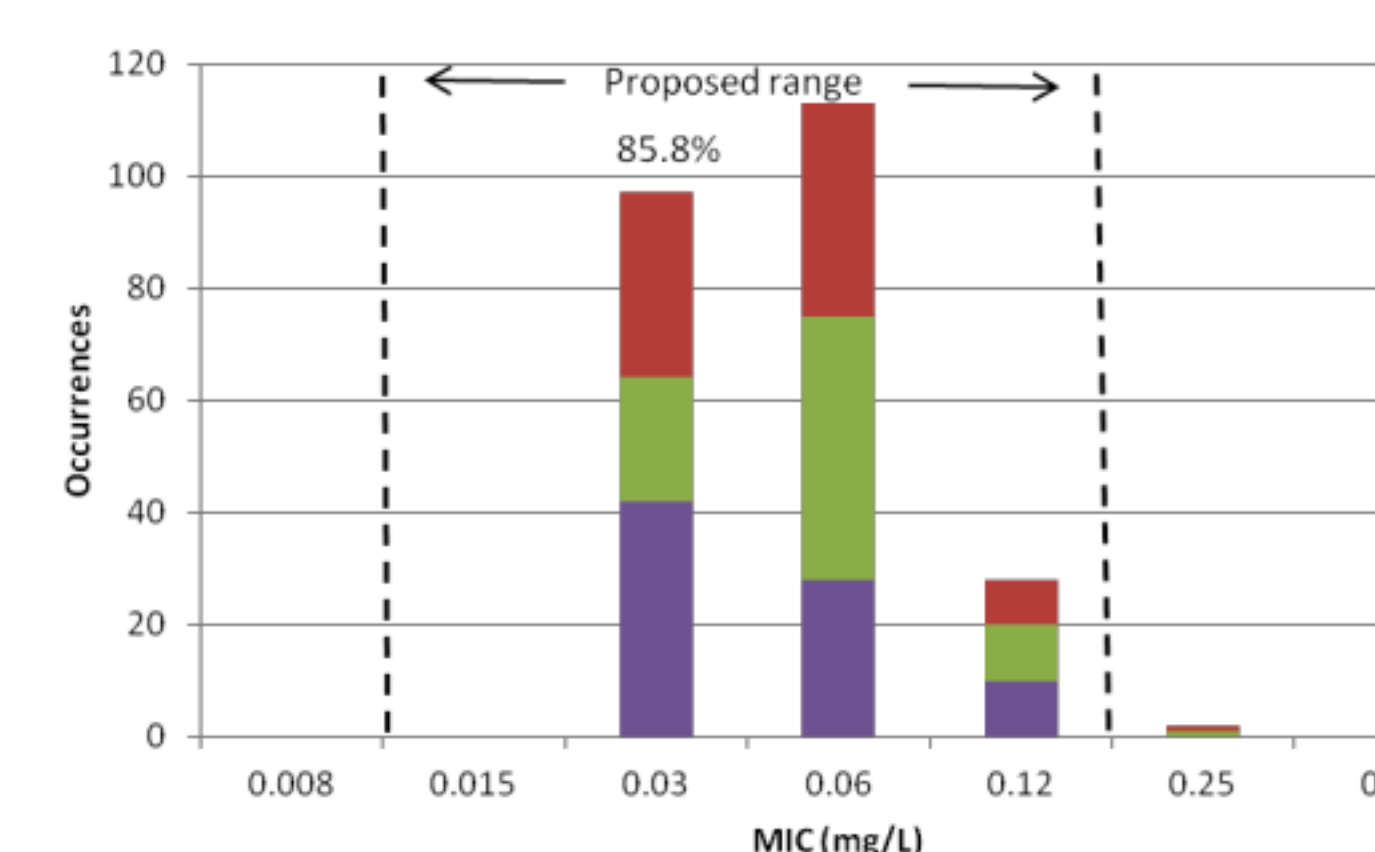


FIGURE 2. Biapenem/RPX7009 Fixed 8 mg/L MIC Distributions for *K. pneumoniae* ATCC BAA1705.



Conclusions

- The proposed QC ranges for broth microdilution methods showed that biapenem, biapenem/RPX7009 at fixed 4 mg/L, and biapenem/RPX7009 at fixed 8 mg/L have generally good inter- and intra-laboratory reproducibility for the commonly utilized control strains: *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and ATCC 35218, *K. pneumoniae* ATCC 700603 and ATCC BAA1705 and *P. aeruginosa* ATCC 27853 (see Table 1).
- These proposed ranges were presented to the CLSI QC working group in June 2012 and all proposed ranges were approved by the AST subcommittee as stated here and to be published in future documents.
- This study established QC ranges that can be utilized to support accurate testing for susceptibility of RPX7009 when combined with biapenem during clinical trials and continued product development.

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