

## Abstract

**Background:** We conducted a study to establish disk diffusion (DD) quality control (QC) ranges for WCK 4282 (cefepime-tazobactam) using a 30/20- $\mu$ g disk (2 manufacturers) and the reference DD method. WCK 4282 is under clinical development for the treatment of serious Gram-negative infections.

**Methods:** An eight laboratory study design followed CLSI M23-A4 guidelines. Five QC strains were tested (*Escherichia coli* ATCC 25922 [EC25922], *E. coli* NCTC 13353 [EC13353], *Klebsiella pneumoniae* ATCC 700603 [KPN700603], *Pseudomonas aeruginosa* ATCC 27853 [PSA27853], and *Staphylococcus aureus* ATCC 25923 [SA25923]) using three agar lots (three manufacturers). Ten replicate tests/disk lot/media lot/site were performed for each QC organism generating 480 zone diameters/QC strain (2,400 total results). Cefepime and piperacillin-tazobactam were used as control agents.

**Results:** The zone diameter QC range for cefepime-tazobactam disk test from eight laboratories when testing EC25922 was 32 – 37 mm, which included 97.9% of the reported results. (See **Table 1**). EC13353 is a CTX-M-15 producer and was included to properly evaluate tazobactam inhibition effect. The proposed zone diameter QC range of 27 – 31 mm for EC13353 included 96.7% of results. The KPN700603 strain, a SHV-18 producer, provided a six mm QC range of 25 – 30 mm with 99.4% of the results included. Another narrow range was proposed for PSA27853 of 27 – 31 mm which included 97.3% of all reported zone diameters. The SA25923 QC strain demonstrated a range of 24 – 30 mm, which included 99.0% of reported results. Using Range Finder statistical program, there were no laboratories or media lot identified as an outlier. All cefepime and piperacillin-tazobactam disk zones generated for the internal controls were within the CLSI published QC ranges.

**Conclusions:** These disk QC ranges for WCK 4282 (cefepime-tazobactam) should accurately guide clinical and reference laboratories participating in the testing of clinical trial isolates and facilitate the regulatory review process for this investigational antimicrobial combination.

## Introduction

WCK 4282 (high-dose cefepime-tazobactam) is a new antibacterial combination consisting of the  $\beta$ -lactamase inhibitor tazobactam combined with the fourth-generation cephalosporin, cefepime. It has demonstrated excellent antibacterial activity against contemporary Gram-negative pathogens including isolates showing resistance to existing drug classes. A Clinical and Laboratory Standards Institute (CLSI) M23-style (tier 2) quality control (QC) study was performed to establish disk diffusion QC ranges for cefepime-tazobactam (30/20- $\mu$ g disks) against five reference QC bacterial strains. These ranges will assist clinical and reference laboratories in monitoring the activity of this combination during clinical trials and in clinical microbiology practice.

## Methods

**Participating institutions:** A total of eight laboratories participated in the CLSI M23 QC study and provided WCK 4282 (cefepime-tazobactam) disk diffusion data for the QC reference strains, as follows: JMI Laboratories, North Liberty, Iowa, USA (R.N. Jones, M.D.); University of Rochester, Rochester, New York, USA (D. Hardy, Ph.D.); TREK Diagnostic Systems/ThermoFisher Scientific, Cleveland, Ohio, USA (C. Knapp, M.S.); University of Alberta Hospitals, Edmonton, Alberta, Canada (R. Rennie, Ph.D.); Marquette University, Milwaukee, Wisconsin, USA (E. Munson, Ph.D.); Indiana University Health Methodist Hospital, Indianapolis, Indiana, USA (G. Denys, Ph.D.); Cleveland Clinic Foundation, Cleveland, Ohio, USA (G. Procop, M.D.) and Henry Ford Hospital, Detroit, Michigan, USA (M. Zervos, M.D.).

**Susceptibility testing:** Disk diffusion susceptibility testing was conducted using three lots of Mueller-Hinton (MH) agar plates produced by Hardy Diagnostics (Santa Maria, California, USA), Remel (Lenexa, Kansas, USA), and BBL (Becton Dickinson; Franklin Lakes, New Jersey, USA). Cefepime-tazobactam (30/20- $\mu$ g) disk lots were manufactured by Bio-Rad Laboratories (Hercules, California, USA) and the MAST Group Ltd (Bootle, Merseyside, United Kingdom) whereas cefepime (30- $\mu$ g) and piperacillin-tazobactam (100/10- $\mu$ g) control disks were produced by Becton Dickinson (BD; Franklin Lakes, New Jersey, USA). Disk diffusion zone of inhibition testing was performed as described in CLSI document M02-A12 (2015). Inoculated MH agar plates were incubated for 16-18 hours at 35°C in an ambient air incubator prior to reading the zones of inhibition.

Appropriate starting inoculum concentrations were verified by performing colony counts. The QC reference strains tested included: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and NCTC 13353, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. Ten replicates of each QC strain were tested using three different lots of MH agar and two lots of cefepime-tazobactam (30/20- $\mu$ g) disks obtained from two separate manufacturers in eight distinct qualified laboratories generating 480 zone diameters for each QC reference strain. Similarly, a total of 240 zone diameters were generated for cefepime and piperacillin-tazobactam control disks against each of the QC reference strains.

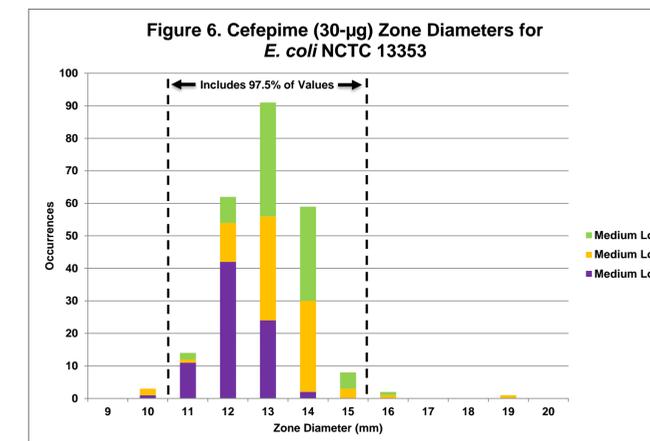
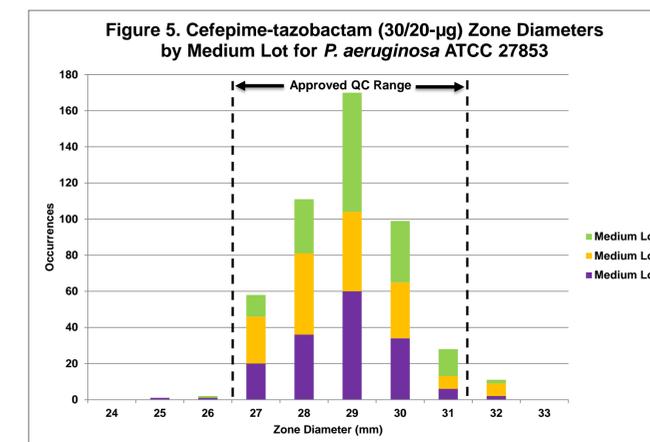
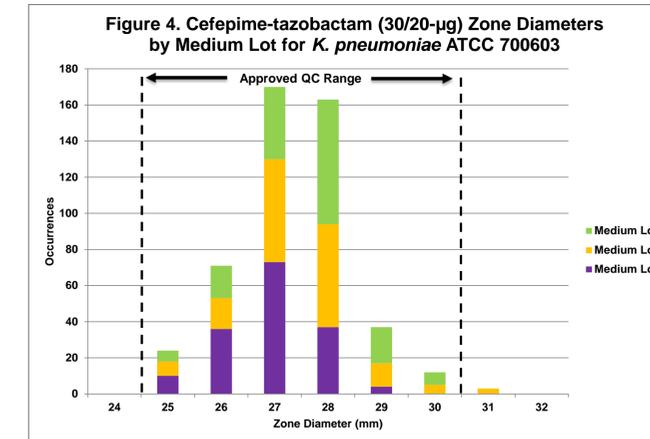
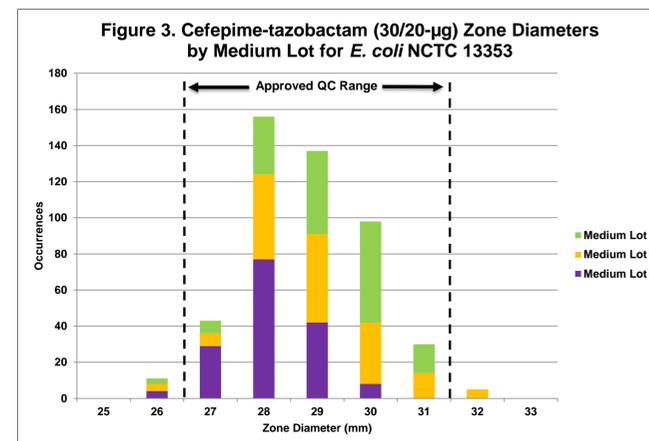
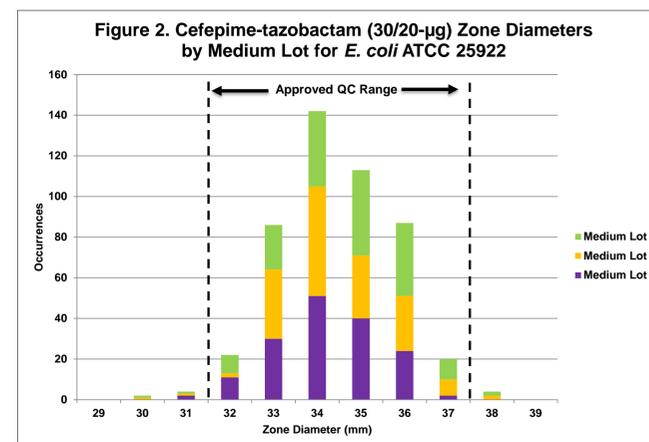
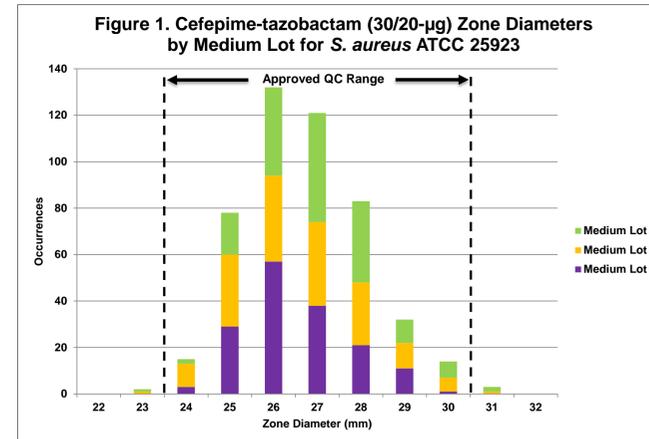
## Results

- Applying CLSI M23-A4 analysis criteria to cefepime-tazobactam 30/20- $\mu$ g disks, 96.7 – 99.4% of the zone of inhibition results from the eight participating laboratories were within the proposed QC ranges recently approved (CLSI meeting; January, 2016) for each of the five quality control reference strains tested (**Table 1** and **Figures 1-5**).
- E. coli* NCTC 13353 (CTX-M-15 producing strain) was effective as a QC strain in demonstrating the  $\beta$ -lactamase inhibition activity of tazobactam in the cefepime-tazobactam combination (**Figures 3** and **6**).
- Zone of inhibition results for the cefepime (720/720; 100.0%) and piperacillin-tazobactam (720/720; 100.0%) control disks were all within CLSI published QC ranges, providing validated internal controls for this study.
- Among the three lots of MH agar tested, median zones of inhibition using Media lot C (BBL) were generally  $\leq 1$  mm smaller than zone of inhibition values obtained with media lots A or B.

**Table 1. Proposed/Approved CLSI quality control ranges for WCK 4282 (cefepime-tazobactam; 30/20- $\mu$ g) disk diffusion testing**

QC reference strain	Disk diffusion zone diameters for WCK 4282 (cefepime-tazobactam; 30/20- $\mu$ g)	
	Proposed/Approved Range mm (% in range)	Proposed Range Finder Range mm (% in range)
<i>S. aureus</i> ATCC 25923	24 – 30 (99.0%)	23 – 30 (99.4%)
<i>E. coli</i> ATCC 25922	32 – 37 (97.9%)	31 – 38 (99.6%)
<i>E. coli</i> NCTC 13353 <sup>a</sup>	27 – 31 (96.7%)	26 – 32 (100.0%)
<i>K. pneumoniae</i> ATCC 700603	25 – 30 (99.4%)	25 – 30 (99.4%)
<i>P. aeruginosa</i> ATCC 27853	27 – 31 (97.3%)	26 – 32 (99.8%)

a. This CTX-M-15 producing strain is needed to evaluate tazobactam for  $\beta$ -lactamase inhibition.



## Conclusions

- WCK 4282 (cefepime-tazobactam; 30/20- $\mu$ 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 and *P. aeruginosa* ATCC 27853.
- Good inter- and intra-laboratory reproducibility was also observed for cefepime and cefepime-tazobactam disk diffusion results against *E. coli* NCTC 13353 (CTX-M-15). This supplementary QC reference strain is needed to evaluate the tazobactam component of the cefepime-tazobactam combination for  $\beta$ -lactamase inhibition.
- This study established disk diffusion QC ranges for WCK 4282 (cefepime-tazobactam) against five QC reference strains that can be utilized to support accurate antimicrobial susceptibility testing.
- The CLSI subcommittee on Antimicrobial Susceptibility Testing approved WCK 4282 (cefepime-tazobactam) disk diffusion QC ranges for each of these five reference strains in January, 2016.

## Acknowledgements

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## References

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