

# Proposed Revised Disk Diffusion Quality Control Ranges for Doripenem Versus *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853

## #E-1525

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## Revised Abstract

**Background:** The CLSI publishes recommendations for quality control (QC) of antimicrobial susceptibility tests. Since these initial approved QC ranges are based on a limited number of media lots, drug lots, and testing laboratories, sometimes it is necessary to revise the ranges after accumulation of data from multiple laboratories, media lots, and disk lots. Doripenem disk diffusion QC ranges were approved several years ago by CLSI, but subsequent data collected during routine testing by hundreds of clinical labs showed many unable to successfully complete 20- or 30-day QC regimens, with zone diameters for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 smaller than those initially approved. This study was done to determine if the ranges required adjustment.

**Methods:** Following CLSI M23 guidelines, each of 3 independent laboratories tested 28 replicates of *E. coli* 25922 and *P. aeruginosa* 27853 using 2 lots of Mueller-Hinton agar (1 from each of 2 manufacturers) and 3 lots of doripenem disks (1 from each of 3 manufacturers). Meropenem was used as the control. Each replicate used a unique inoculum preparation which was verified by colony count. QC ranges were determined following M23 requirements.

**Results:** Although 96% of *E. coli* zones were within the current CLSI QC range of 28-35mm, 74% were in the lower half of the range, and 11% and 19% of zones fell at 28 and 29mm, respectively. For *P. aeruginosa*, 99% of doripenem zones were within the current CLSI QC range of 29-35mm; however, 63% were in the lower half of the range, and 6% and 22% of zones fell at 29 and 30mm, respectively.

**Conclusions:** Using the CLSI M23 method for determining QC ranges (mean +/- 1.96 standard deviations), both *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 had their ranges lowered by one millimeter in the CLSI M100-S20 document published in January 2010; however the upper end of the ranges remained unchanged to encompass the original QC data as well as those from this study, while still having acceptably narrow (8-9mm) ranges.

## Introduction

Quality control ranges for disk diffusion testing of doripenem were established by the Clinical and Laboratory Standards Institute (CLSI) several years ago. Doripenem was approved for market October 2007. During the past 1-2 years, International Health Management Associates, Inc. (IHMA) has been providing doripenem disks to many laboratories which did not have this drug on their routine susceptibility testing systems (MicroScan, Vitek, etc.). During this program, IHMA received many notifications from labs which were unable to successfully complete their 30-day QC testing with this drug due to numerous out-of-control (low) zone measurements with *P. aeruginosa* ATCC 27853 and, to a lesser but still significant extent, with *E. coli* ATCC 25922. No problems were reported with the other CLSI-recommended QC strains.

IHMA worked closely with disk and Mueller-Hinton agar manufacturers to try to determine the reason(s) for these problems, but was unable to attribute the smaller zone sizes to any factor such as lot of medium, disk manufacturer, incubation conditions, or inoculum preparation. Having ruled out technique or product issues, we believed that perhaps the original zone ranges established several years ago needed to be revisited and possibly changed to reflect the real world experience of the past few years. Toward this end, J&J Pharmaceutical Research and Development sponsored an M23 Tier 3 study to reassess the QC zone diameters for doripenem vs. *E. coli* 25922 and *P. aeruginosa* 27853 as previously published [1].

## Materials & Methods

### M23-A3 Guidelines

The study followed CLSI M23-A3 requirements for Tier 3 QC studies [2]:

- 3 laboratories/independent sites;
- 2 media lots (different manufacturers);
- at least 10 replicates of each QC strain per laboratory (at least 50 total test points per medium lot);
- at least 2 lots of disks (different manufacturers);
- at least 500 test points per QC strain/drug combination

The three participating laboratories in the Tier 3 study were:

1. Laboratory Specialists Inc., Westlake, OH (Laura Koeth, President)
2. Trek Diagnostic Systems, Cleveland, OH (Cindy Knapp, Director Laboratory Services)
3. International Health Management Associates, Inc., Schaumburg, IL (Dr. Daryl Hoban, Director; Dr. Meredith Hackel, Assoc. Director)

### Compounds

Mueller-Hinton agar plates (150mm) were purchased from two manufacturers: BD (Franklin Lakes, NJ) and Remel (Lenexa, KS). Meropenem control disks (10 mg) were purchased from BD, and doripenem disks (10 mg) were purchased from three manufacturers: Oxoid (Cambridge, UK), BD, and Mast Diagnostics (Merseyside, UK). Supplies were distributed by IHMA to the other two sites.

### Antimicrobial Testing

Each laboratory prepared four separately-adjusted inocula of each of the two QC strains on each of seven days; each inoculum was streaked onto two 150mm Mueller-Hinton agar plates—one from each of the two media manufacturers. Each plate had 3 doripenem disks, one from each of three disk manufacturers. A meropenem disk was used as a control. All inoculum densities were validated with colony counts, plates were incubated and zone diameters read following CLSI guidelines [2]. If meropenem zone sizes were out of control on any given day, that day's doripenem data were to be discarded.

## References

1. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100-S19, 2009. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
2. Clinical and Laboratory Standards Institute, Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline - Third Edition. CLSI document M23-A3, 2007. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA.
3. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100-S20, 2010. Clinical and Laboratory Standards Institute. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA.

## Results

Table 1. *E. coli* ATCC 25922 doripenem results by lab (3 disks, 2 MHA lots); modes are yellow-highlighted.

Zone (mm)	Lab 1	Lab 2	Lab 3	All Labs
25				
26		3		3
27	1	16		17
28	23	35		58
29	62	37		99
30	91	35	14	130
31	40	15	49	104
32	13	11	72	96
33	2	2	15	19
34		2		2
35				0
36				
37				
38				
% in range	99.5%	87.8%	100.0%	96.2%

Table 2. *E. coli* ATCC 25922 doripenem results by disk manufacturer (2 MHA lots, 3 labs); modes are yellow-highlighted.

Zone (mm)	Disk A	Disk B	Disk C	All Disks
25				
26			2	2
27	3	7	7	17
28	13	27	18	58
29	25	38	36	99
30	46	39	45	130
31	37	25	42	104
32	32	27	37	96
33	16	2	1	19
34	2			2
35				
36				
37				
38				
% in range	98.3%	96.0%	94.9%	96.4%

Table 3. *E. coli* ATCC 25922 doripenem results by MHA lot (3 disk lots, 3 labs); modes are yellow-highlighted.

Zone (mm)	MHA 1	MHA 2	Both MHA
25			
26	0	3	3
27	5	12	17
28	21	37	58
29	40	59	99
30	66	64	130
31	49	55	104
32	65	31	96
33	17	2	19
34	1	1	2
35	0	0	0
36			
37			
38			
% in range	98.1%	94.3%	96.2%

Final *E. coli* inoculum concentrations ranged from  $4.0 \times 10^7$  to  $2.03 \times 10^8$ . 100% of the control drug meropenem zones were within the CLSI range of 28-34mm (data not shown). Although significant differences were observed between doripenem zone sizes measured on the two lots of agar ( $p < 0.0001$ ), in practical terms the differences were small, with means of 30.5 and 29.8mm. Slightly different zones were observed between the three lots/manufacturers of disks for this QC strain, with disk A being significantly different from B and C ( $p < 0.0001$ ), whereas B and C were not different from each other ( $p = 0.28$ ). Statistically significant differences were seen when comparing zones generated by the three labs ( $p < 0.0001$  for all comparisons), with Lab 3 having a mean zone diameter 2.3mm larger than Lab 2.

96.4% of doripenem zones were within the current CLSI QC range of 28-35mm; 11% and 19% of zones fell at 28 and 29mm, respectively; 74.1% were in the lower half of the range. Applying the calculation of [mean +/- 1.96 SD] to these Tier 3 data suggested a reduction of 1mm to the range, lowering it from 29-35mm to 28-34mm.

Figure 1. *E. coli* ATCC 25922 doripenem results by lab (3 disks, 2 MHA lots)

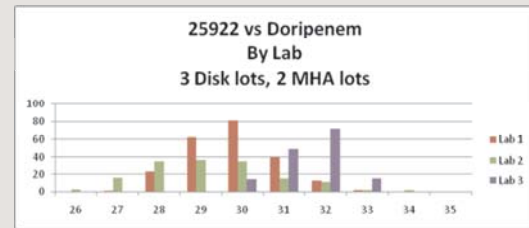


Figure 2. *E. coli* ATCC 25922 doripenem results by disk manufacturer (2 MHA lots, 3 labs).

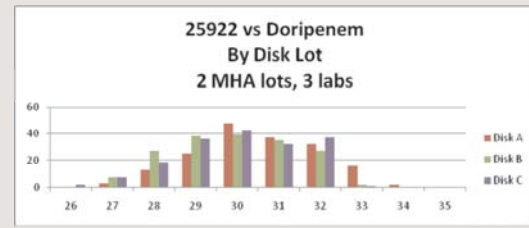


Figure 3. *E. coli* ATCC 25922 doripenem results by MHA lot (3 disk lots, 3 labs).

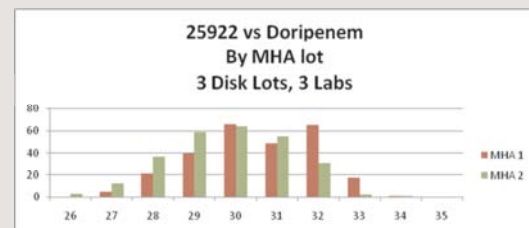


Table 4. *P. aeruginosa* ATCC 27853 doripenem results by lab (3 disks, 2 MHA lots); modes are yellow-highlighted.

Zone (mm)	Lab 1	Lab 2	Lab 3	All Labs
25				
26				
27	0	0	0	0
28	1	2	0	3
29	25	4	0	29
30	74	17	26	117
31	89	38	56	183
32	30	48	64	142
33	3	35	4	42
34	0	12	0	12
35	0	0	0	0
36				
37				
38				
% in range	99.5%	98.7%	100.0%	99.4%

Table 5. *P. aeruginosa* ATCC 27853 doripenem results by disk manufacturer (2 MHA lots, 3 labs); modes are yellow-highlighted.

Zone (mm)	Disk A	Disk B	Disk C	All Disks
25				
26				
27	0	0	0	0
28	0	1	2	3
29	6	7	22	29
30	15	39	63	117
31	65	63	55	183
32	57	54	31	142
33	27	12	3	42
34	12	0	0	12
35	0	0	0	0
36				
37				
38				
% in range	100.0%	99.4%	98.9%	99.4%

Table 6. *P. aeruginosa* ATCC 27853 doripenem results by MHA lot (3 disk lots, 3 labs); modes are yellow-highlighted.

Zone (mm)	MHA 1	MHA 2	Both MHA
25			
26			
27	0	0	0
28	1	2	3
29	15	14	29
30	57	60	117
31	90	93	183
32	69	73	142
33	25	17	42
34	7	5	12
35	0	0	0
36			
37			
38			
% in range	99.6%	99.2%	99.4%

Final *P. aeruginosa* inoculum concentrations ranged from  $1.0 \times 10^8$  to  $2.21 \times 10^8$ . 100% of the control drug meropenem zones were within the CLSI range of 27-33mm (data not shown). No medium differences were observed ( $p = 0.4$ ). Although there was statistically significant variability seen between labs ( $p < 0.0001$ ) and between disk manufacturer ( $p < 0.0001$ ), the maximum difference in mean zone sizes between labs and among disk was only 1.2mm.

99.4% of doripenem zones were within the current CLSI QC range of 29-35mm; 5.5% and 22.0% of zones fell at 29 and 30mm, respectively; and 62.9% were in the lower half of the range. Applying the calculation of [mean +/- 1.96 SD] to these Tier 3 data suggested a reduction of 1mm to the range, lowering it from 29-35mm to 28-34mm.

Figure 4. *P. aeruginosa* ATCC 27853 doripenem results by lab (3 disks, 2 MHA lots).

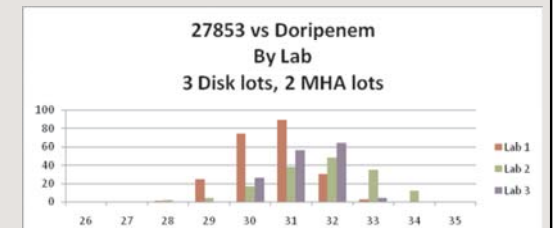


Figure 5. *P. aeruginosa* ATCC 27853 doripenem results by disk manufacturer (2 MHA lots, 3 labs).

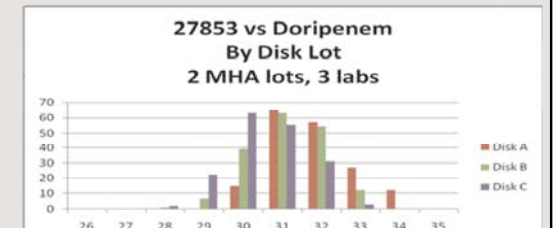
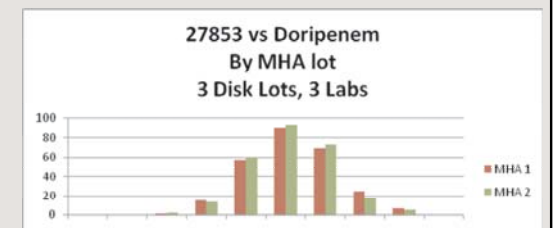


Figure 6. *P. aeruginosa* ATCC 27853 doripenem results by MHA lot (3 disk lots, 3 labs).



## Conclusions

Using the CLSI M23 method for determining QC ranges (mean +/- 1.96 standard deviations), both the *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 ranges were lowered by one millimeter in the CLSI M100-S20 document published in January 2010 [3]; however the upper end of the ranges remained unchanged to encompass the original QC data as well as those from this study, while still having acceptably narrow (8-9mm) ranges.

### Quality Control Zone Diameters for Doripenem:

Control Strain	Original Range	New Range 2010
<i>E. coli</i> ATCC 25922	28-35	27-35
<i>P. aeruginosa</i> ATCC 27853	29-35	28-35