MONDAY-020

CLSI M23 Tier 2 Study to Establish Disk Diffusion Quality Control Ranges for S-649266

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Revised Abstract

Background: S-649226 is a novel parenteral siderophore cephalosporin with potent activity against Gram-negative pathogens including carbapenem-resistant isolates and is currently in clinical development with Shionogi & Co., Ltd. This study was performed to establish quality control (QC) disk diffusion ranges for S-649266 against specific ATCC QC organisms utilized by the Clinical and Laboratory Standards Institute (CLSI). **Methods**: Two ATCC QC organisms within the spectrum of S-649266 activity were tested: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. S-649266 and a control agent (cefepime) were tested against both QC strains following CLSI guidelines for disk diffusion. This study design met the CLSI M23-A3 requirements for Tier 2 QC studies: seven laboratories/independent sites; three media lots (different manufacturers); two disk lots; 10 replicates of each QC strain per laboratory (at least 70 total test points per medium lot); and at least 210 test points per QC strain/drug combination. Calculation of the proposed QC ranges was based on the Gavan statistic. **Results**: Ranges approved by the CLSI are shown in the table. All cefepime results were within the established CLSI ranges.

Summary of Approved CLSI M23 Tier 2 Quality Control Ranges for S-649266

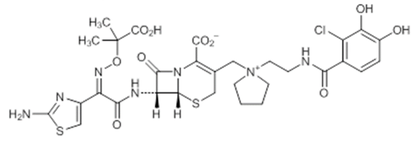
Organism	Proposed QC Range (mm)	Size of range	(%) in Range
E. coli ATCC 25922	23-31	9	95.3 (515/540)
P. aeruginosa ATCC 27853	20-30	11	94.5 (397/420)

Conclusions: Using M23-A3 criteria, a nine millimeter range of 23 – 31 mm included 95.3% of all reported results for *E. coli* ATCC 25922. The Gavan statistic calculated a 13 millimeter range of 19 – 31 mm for *P. aeruginosa* ATCC 27853, which included 95.0% of all reported results. It was noted that a significant number of test results for *P. aeruginosa* ATCC 27853 on Media C were at the higher end (larger zones) of the calculated range. Under the guidance of the CLSI Quality Control Working Group, further analysis was performed, eliminating data from two of the laboratories. This analysis resulted in an 11 mm range of 20-30 mm, including 94.5% of all reported results, which was approved by the CLSI in January 2016.

Introduction

S-649226 is a novel parenteral siderophore cephalosporin with potent activity against Gram-negative pathogens including carbapenem-resistant isolates and is currently in clinical development with Shionogi & Co., Ltd. This study was performed to establish quality control (QC) disk diffusion ranges for S-649266 against specific ATCC QC organisms utilized by the Clinical and Laboratory Standards Institute (CLSI). This study design met or exceeded the following CLSI M23-A3 requirements for Tier 2 QC studies: seven laboratories/independent sites; three media lots (different manufacturers); two disk lots; at least 10 replicates of each QC strain per laboratory (at least 70 total test points per medium lot); and at least 210 test points per QC strain/drug combination [1].

Figure 1. Chemical Structure of S-649266



Materials & Methods

- A multi-laboratory (9 laboratories) study to evaluate QC ranges for disk diffusion was carried out according to CLSI guidelines [1].
- Two ATCC QC organisms within the spectrum of S-649266 activity were tested: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.
- S-649266 and a control agent (cefepime) were tested against both QC strains following CLSI guidelines [2, 3]. All cefepime results were within CLSI-approved ranges.
- Two lots of S-649266 disks were manufactured by MAST (Merseyside, UK) and Bio-Rad (Marnes-la-Coquette, FR) were tested on three lots of agar plates.
- Supplies, including agar plates and disks were distributed by IHMA, Inc. (Schaumburg, IL, US) to the other eight sites.

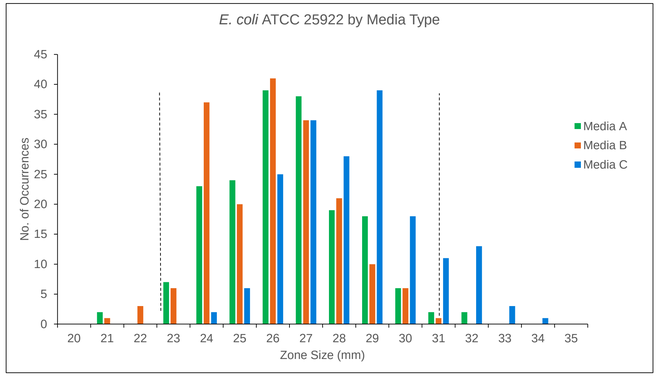
Results

- ***E. coli* ATCC 25922**
  - **The greater than 95% within range CLSI criterion was met by all data from all nine laboratories (Table 1, Figure 1).**
  - **There were no significant differences in disk diffusion results between the two different lots of S-649266 disks or the three lots of agar.**

Table 1. Media lot, disk lot, and inter- and intra-laboratory comparisons of S-649266 disk diffusion results versus *E. coli* ATCC 25922 Lot (CLSI-approved QC range shown by dotted lines).

Zone	Occurrences By Media Lot			Occurrences by disk lot		Laboratory Code Occurrences)									Total N
(mm)	A	B	C	A	B	A	B	C	D	E	F	G	H	I	
19															0
20															0
21	2	1		3									3		3
22		3		3									3		3
23	7	6		12	1	5		1			3		3	1	13
24	23	37	2	57	4	14		15	1		9	10	9	3	61
25	24	20	6	46	7	9	4	5	3	2	7	9	4	10	53
26	39	41	25	59	44	14	7	15	5	11	12	16	13	10	103
27	38	34	34	41	60	9	6	14	7	9	17	20	4	15	101
28	19	21	28	20	44	5	5		13	10	4	3	12	12	64
29	18	10	39	18	54	3	14	10	12	15	6	2	2	8	72
30	6	6	18	9	24	1	9		11	4	2		5	1	33
31	2	1	11	2	13		7		2	4			2		15
32	2		13		15		6		4	5					15
33			3		3		2		1						3
34			1		1				1						1
35															0
Total	180	180	180	270	270	60	60	60	60	60	60	60	60	60	540
Median	26	26	28	26	28	26	29	26	29	28	26	26	26	27	27
Mode	26	26	29	26	27	24	29	24	28	29	27	27	26	27	26
GeoMean	26.3	26.0	28.3	25.8	28.1	25.6	28.8	26.0	28.5	28.2	26.2	26.0	26.0	26.8	26.9
Range	12	11	11	11	12	8	9	7	11	8	8	6	11	8	

Figure 1. S-649266 Disk Diffusion Zone Distribution for *E. coli* ATCC 25922 by Media Lot (CLSI-approved QC range shown by dotted lines).



- ***P. aeruginosa* ATCC 27853**
  - **Using M23-A3 criteria, a 13 mm range of 19 – 31 mm included 95.0% of all reported results (Table 1, Figure 2). It was noted that a significant number of test results from Media C were at the higher end (larger zones) of the calculated range.**
  - **To confirm that this was not limited to one lot of Media C, two additional lots of each media were tested (Figure 3). Again, a significant number of test results from the two new lots of Media C produced larger zones compared to the new lots of Media A and B.**
  - **The original data analysis was repeated excluding data from two labs that provided many of the larger zone sizes from Media C. This analysis, which met CLSI M23 criteria, resulted in an 11 mm range of 20 – 30 mm including 94.5% of all reported results (Figure 4). This range was approved by the CLSI committee with the following suggestions:**
    - **An attempt should be made to determine the iron content in the agar tested.**
    - **When conducting clinical trials and future surveillance studies utilizing disk diffusion, media type should be monitored and quality control ranges should also be carefully recorded and monitored.**
    - **Additional disk data, both internal and from clinical trials, should be collected and presented at the June 2016 CLSI meeting.**

Table 2. Media lot, disk lot, and inter- and intra-laboratory comparisons of S-649266 disk diffusion results versus *P. aeruginosa* ATCC 27853, all laboratories, all media lots.

Zone	Occurrences By Media Lot			Occurrences by disk lot		Laboratory Code (Occurrences)									Total N
(mm)	A	B	C	A	B	A	B	C	D	E	F	G	H	I	
16															0
17		1		1									1		1
18		9		9									9		9
19	3	4		7			4					3			7
20	10	14		24		3	2	1				6	6	6	24
21	15	32		44	3	6	2	8	4	4	2	5	7	9	47
22	18	31		43	6	2	8	5	4	9	8	5	3	5	49
23	25	15	1	28	13	7	5	1	4	5	6	6	5	2	41
24	34	31	2	24	43	11	4	5	11	8	5	9	4	10	67
25	20	34	8	11	51	4	4	10	6	7	8	11	7	5	62
26	23	7	20	19	31	12	4	7	5	4	4	4	5	5	50
27	24	2	24	22	28	4	4	8	5	8	12	3	4	2	50
28	5		30	20	15	3	4	4	2	3	5	5	2	7	35
29	3		32	9	26	7	5	7	4	5	1	1	3	2	35
30			33	5	28	1	6	4	3	3	8	2	4	2	33
31			13	3	10		3		2	3	1			4	13
32			8	1	7		1		5	1				1	8
33			6		6		2		4						6
34			3		3		2		1						3
35															0
Total	180	180	180	270	270	60	60	60	60	60	60	60	60	60	540
Median	24	22	29	23	26	25	26	25.5	26	25	26	24	23	24	25
Mode	24	25	30	21	25	26	22	25	24	22	27	25	18	24	24
GeoMean	24.0	22.5	28.6	23.3	26.6	24.7	25.6	25.2	26.3	25.3	25.6	23.8	23.0	24.5	24.9
Range	11	11	12	16	14	11	16	11	14	12	11	12	14	13	

Figure 2. S-649266 Disk Diffusion Zone Distribution for *P. aeruginosa* ATCC 27853 by Media Lot, All Nine Laboratories (CLSI-approved QC range shown by dotted lines).

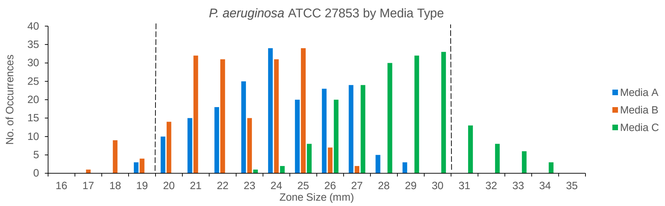


Figure 3. S-649266 Disk Diffusion Zone Distribution for *P. aeruginosa* ATCC 27853 Versus Six MHA Lots Performed by a Single Laboratory (CLSI-approved QC range shown by dotted lines).

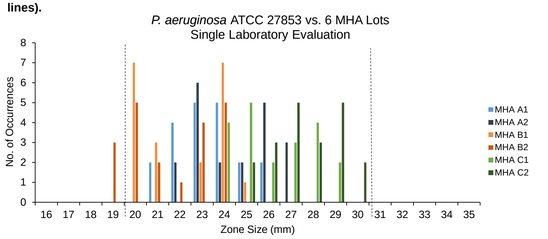
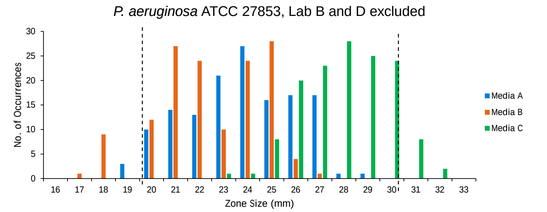


Figure 4. S-649266 Disk Diffusion Zone Distribution for *P. aeruginosa* ATCC 27853 for Seven Laboratories.



Conclusions

The following disk diffusion ranges for S-649266 were accepted by the CLSI at the January, 2016 meeting with the recommendation that additional data be collected and presented at the June 2016 CLSI meeting:

	Disk Diffusion	
Organism	Proposed QC Range (mm)	Percent in Range
E. coli ATCC 25922	23 - 31	95.3
P. aeruginosa ATCC 27853	20 - 30	94.5

References

1. Clinical and Laboratory Standards Institute. 2007. *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline - Third Edition*. CLSI document M23-A3. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

2. Clinical and Laboratory Standards Institute. 2015. *Performance Standard for Antimicrobial Disk Susceptibility Tests; Approved Standards - Twelfth Edition*. CLSI document t M02-A12. Wayne, Pennsylvania 19087-1898 USA.

3. Clinical and Laboratory Standards Institute. 2015. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement*. CLSI Document M100-S25. Wayne, Pennsylvania 19087-1898 USA.