

Abstract

Background: Solithromycin is a novel fluoroketolide undergoing Phase 2 trials for the treatment of community-acquired pneumonia. With any new agent, it is important to have available reliable and reproducible methods of evaluating *in vitro* activity during development and post-approval. Solithromycin disks zones correlate well with broth microdilution MICs demonstrating the feasibility of disk diffusion testing of solithromycin. This study establishes quality control ranges to be used for disk diffusion susceptibility testing of solithromycin during development and post-approval.

Methods: Quality control ranges for the disk diffusion testing of solithromycin disks (15 micrograms) were determined per CLSI guidelines (M23-A3). 9 separate laboratories each tested 10 independent replicates of each relevant ATCC QC organism (*S. aureus* ATCC 25923, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247) across three lots of media from different manufacturers against two disk lots of solithromycin (one from MAST and one from BioRad). One disk lot of telithromycin was tested as an in class control. Statistical methods (Gavin statistic and Rangefinder) were used to evaluate the data and generate ranges suitable for quality control testing.

Results: Recently approved CLSI QC ranges for the disk diffusion testing of solithromycin and their overall performance are shown in the table 1. These ranges take into account intra-laboratory, intra-media lot, and intra-disk lot variation.

Conclusions: The proposed ranges are suitable for quality control testing of solithromycin disks at the indicated disk mass. The performance of these ranges should be continually monitored during disk testing that takes place as part of the clinical development of solithromycin going forward.

- Zone ranges presented here were approved by CLSI during the June 2011 meeting (TABLE 1)
 - 97.0% of the results obtained *S. aureus* ATCC 25923 were within the approved 22 – 30 mm zone range
 - 99.4% of the results obtained for *S. pneumoniae* ATCC 49619 were within the approved 25-33 mm zone range
 - 97.6% of the results obtained for *H. influenzae* ATCC 49247 were within the approved 16-23 mm zone range
 - Data represents results obtained when the control drug telithromycin was within the current CLSI approved ranges (data not shown)

- Approved ranges were similar to those returned from analysis of central tendency statistics by both Gavin Statistic and Rangefinder excluding *S. pneumoniae* where the distribution itself justified a wider zone (TABLE 2)

- For all evaluated organisms, there was little inter-laboratory variation in zone size distribution, with the majority of results falling within the approved ranges (TABLES 3-5, FIGURES 1-3)

- For all evaluated organisms, there was little variation in zone size distribution across media lots and across disk lots (TABLES 3-5, FIGURES 1-3)

TABLE 1. CLSI approved QC ranges for the disk diffusion testing of Solithromycin.

Organism	QC range (mm)	# mm	Total	
			Results (N)	n (%) in range
<i>S. aureus</i> ATCC 25923*	22 - 30	9	540	522 (96.7)
			536 ^b	520 (97.0)
<i>S. pneumoniae</i> ATCC 49619	25-33	9	540	536 (99.3)
			532 ^b	529 (99.4)
<i>H. influenzae</i> ATCC 49247	16 – 23	8	540	527 (97.6)

^aIn the instance of double zones, diameter of inner zones were reported
^bSolithromycin results for replicates where telithromycin control was out of QC removed from analysis

TABLE 2. Central tendency statistical analysis of data by Gavin Statistic and Rangefinder

<i>S. aureus</i> ATCC 25923	<i>S. pneumoniae</i> ATCC 49619	<i>H. influenzae</i> ATCC 49247
Gavin statistic: (mm)	Gavin statistic: (mm)	Gavin statistic: (mm)
All Lab median = 28	All Lab median = 28	All Lab median = 28
Median of Ranges (MR) = 6	Median of Ranges (MR) = 6	Median of Ranges (MR) = 6
1/2 MR rounded up (R) = 3	1/2 MR rounded up (R) = 3	1/2 MR rounded up (R) = 3
All Lab Median v.i. R = 22-29	All Lab Median v.i. R = 23-29	All Lab Median v.i. R = 22-29
Rangefinder: 22-30	Rangefinder: 22-30	Rangefinder: 22-30

TABLE 5. Solithromycin Disk Zones Against *H. influenzae* ATCC 49247 Across Laboratories, Media Lots, and Disk Lots

Zone Diameter (mm)	Laboratory (Incidence):									Media Lot Code (Incidence):			Disk Lot Code (Incidence):		Total
	A	B	C	D	E	F	G	H	I	Lot A	Lot B	Lot C	Lot 1	Lot 2	
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	13	10	5	1	18	8	13	25	2	14	17	30	42	76	
19	28	16	11	18	23	16	13	19	25	11	17	31	34	41	
20	15	13	20	20	4	17	13	19	28	1	2	40	35	35	
21	4	7	7	1	10	10	8	11	8	1	2	38	7	15	
22	2	8	0	0	3	2	7	2	1	1	2	19	2	11	
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
25	60	60	60	60	60	60	60	60	60	180	180	180	270	270	
Total	60	60	60	60	60	60	60	60	60	180	180	180	268	268	
Median	19	20	20	18	19	19	18	20	19	20	19	19	19	20	
Mean	19.2	20.1	20.5	18.3	19.3	19.3	18.3	20.1	19.4	20.3	19.9	19.9	19.9	20.4	
Range	4	9	0	8	6	6	6	6	6	10	7	6	9	19	

Introduction

Solithromycin is an investigational fluoroketolide that is more potent than existing macrolides, and has an extended spectrum of antimicrobial activity

- Active against key bacterial pathogens associated with community-acquired bacterial pneumonia (CABP)
- Generally 8-16 fold more active than azithromycin and 2-4 more active than telithromycin *in vitro*
- Active against mycobacteria, malaria parasites, and bioterrorism organisms
- Currently in Phase 2 clinical trials for CABP

Methods

The study was done according to the criteria as defined in CLSI M23-A3, Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters.

- Each of nine laboratories participated.
- Each site tested three different agar lots.
- Testing was performed for solithromycin generating zone diameter values in millimeters (mm) for two disk lots on three different media lots for 10 replicates (2 x 3 x 10 = 60 results) at nine sites yielding 540 zone diameter results for each organism tested.
- Telithromycin was used as the control drug and was tested for 10 replicates on each lot of media with one disk lot (3 x 10 = 30 results per lab).

Results

TABLE 3. Solithromycin Disk Zones Against *S. aureus* ATCC 25923 Across Laboratories, Media Lots, and Disk Lots

Zone Diameter (mm)	Laboratory (Incidence):									Media Lot Code (Incidence):			Disk Lot Code (Incidence):		Total
	A	B	C	D	E	F	G	H	I	Lot A	Lot B	Lot C	Lot 1	Lot 2	
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
24	8	3	6	6	13	3	3	8	18	7	21	12	33	33	
25	28	16	3	16	3	2	24	5	27	48	50	26	80	44	
26	17	14	8	20	10	16	10	6	23	42	35	46	65	59	
27	7	22	7	12	18	25	6	14	6	39	35	43	43	74	
28	3	14	3	21	17	14	1	24	23	26	29	44	73	79	
29	2	13	7	7	6	5	6	5	12	13	15	26	28	28	
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total	60	60	60	60	60	60	60	60	60	180	176	180	268	268	
Median	25	28	30	26	27	27	25	28	25.5	28	30	27	30	27	
Mean	25	27	28	26	28	27	25	28	25	28	26	25	27	28	
Mode	25	36	20	28	25	27	26	26	24	26	26	21	26	26	
Range	4	6	13	6	6	4	4	6	5	14	17	16	16	19	

TABLE 4. Solithromycin Disk Zones Against *S. pneumoniae* ATCC 49619 Across Laboratories, Media Lots, and Disk Lots

Zone Diameter (mm)	Laboratory (Incidence):									Media Lot Code (Incidence):			Disk Lot Code (Incidence):		Total
	A	B	C	D	E	F	G	H	I	Lot A	Lot B	Lot C	Lot 1	Lot 2	
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	14	13	11	27	10	27	10	10	10	27	30	30	61	46	
27	32	27	1	14	13	20	3	7	31	37	39	81	46		
28	46	5	23	24	26	3	11	8	49	39	37	66	44		
29	2	17	14	12	9	8	22	31	29	30	30	46	44		
30	0	1	8	28	10	11	28	28	29	28	29	40	53		
31	0	2	14	13	13	1	11	14	10	16	24	40	40	40	
32	0	0	0	0	0	0	0	0	0	0	0	0	0		
33	0	0	0	0	0	0	0	0	0	0	0	0	0		
34	0	0	0	0	0	0	0	0	0	0	0	0	0		
35	0	0	0	0	0	0	0	0	0	0	0	0	0		
36	0	0	0	0	0	0	0	0	0	0	0	0	0		
37	0	0	0	0	0	0	0	0	0	0	0	0	0		
38	0	0	0	0	0	0	0	0	0	0	0	0	0		
Total	52	60	60	60	60	60	60	60	172	180	180	268	268		
Median	27	27	28	28	28	27	31	30	28	28.5	28	28	28		
Mean	27	27	28	28	28	27	31	29	28	27	28	28	28		
Mode	27	24	28	27	27	27	28	27	28	28	28	28	28		
Range	6	6	6	6	6	6	6	6	10	11	9	12	11		

Figure 3. Distribution of Solithromycin Disk Zone Results Overall, by Media Lot, and by Disk Lot against *H. influenzae* ATCC 49247 - Dashed Lines Indicate CLSI Approved QC Range

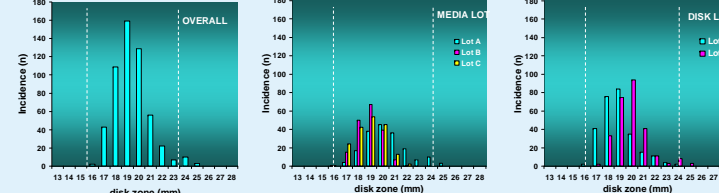


Figure 1. Distribution of Solithromycin Disk Zone Results Overall, by Media Lot, and by Disk Lot against *S. aureus* ATCC 25923 - Dashed Lines Indicate CLSI Approved QC Range

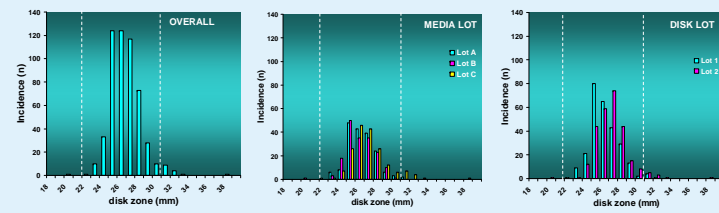
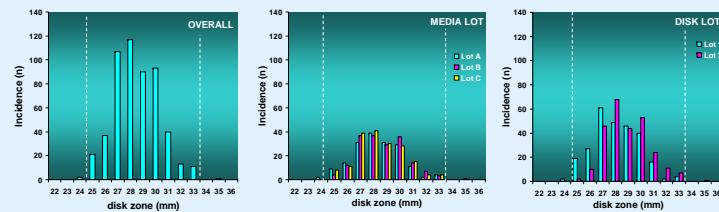


Figure 2. Distribution of Solithromycin Disk Zone Results Overall, by Media Lot, and by Disk Lot against *S. pneumoniae* ATCC 49619 - Dashed Lines Indicate CLSI Approved QC Range



Conclusions

- In accordance with CLSI M23, reliable QC ranges have been established for disk diffusion testing of solithromycin against target respiratory pathogens.
- These ranges will be used going forward to monitor disk test results going during the clinical development of solithromycin and beyond.
- As solithromycin disk zone results continue to be generated, these ranges will be evaluated to determine whether any alteration to these ranges may be necessary

Acknowledgements

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