Solithromycin MIC and Disk Diffusion Quality Control Ranges, a CLSI Multi-Laboratory M23-A3 Study Design

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AMENDED ABSTRACT

Background: Solithromycin is a fluoroketolide with potent activity against most macrolide-resistant pathogens, including *S. pneumoniae*, *N. gonorrhoeae* and other organisms that cause genitourinary tract infections. We conducted two studies to establish MIC quality control (QC) ranges for solithromycin using CLSI reference disk diffusion method and agar dilution methods.

Methods: *N. gonorrhoeae* ATCC 49226 was tested against solithromycin using disk diffusion and agar dilution methods with ciprofloxacin as an internal control. Eight laboratories participated in each study (7 laboratories were the same in both studies). Study designs were compliant with CLSI M23-A3 guidelines with both studies using three media lots of GC agar base. Ten replicates were performed for *N. gonorrhoeae* ATCC 49226 (480 values/disk diffusion; 240 values/agar dilution) against solithromycin.

Results: Proposed QC ranges for *N. gonorrhoeae* ATCC 49226 were established for disk diffusion at 34 – 42mm. This included 95.8% of all reported zone diameters. The Range Finder (RF) statistical program calculated an alternative range of 33 – 43mm which included 98.5% of all zones and was approved by the CLSI subcommittee on Antimicrobial Susceptibility Testing in January 2015. There was no significant difference between the two lots of 15-µg solithromycin disks. The agar dilution study proposed a QC range of 0.03 – 0.25 µg/mL which included all of the solithromycin MIC results. A bimodal "shoulder" (≥60% of data points than the mode) occurs at 0.06 µg/mL creating the need for a four $\log_2$ dilution range. The RF statistical program agreed with this range and found no outlier laboratories. No significant differences were noted among GC agar media lots for solithromycin. All MIC results (80/80; 100.0%) and nearly all disk zones (239/240; 99.6%) of the control agent ciprofloxacin were within CLSI published ranges.

Conclusions: The proposed MIC (0.03 – 0.25 µg/mL) and disk diffusion (33 – 43 mm) QC ranges for solithromycin should guide clinical or reference laboratories participating in the testing of clinical trial isolates and facilitate the regulatory review process for this investigational antimicrobial agents.

INTRODUCTION

Solithromycin (formerly CEM-101) is the first investigational fluoroketolide that recently entered clinical development; and it has shown advantages over other macrolides in its activity against many bacterial pathogens, including *Neisseria gonorrhoeae*. This new macrolide is being developed for oral and intravenous delivery for the treatment of patients with community acquired bacterial pneumonia (CABP) and urethritis. Solithromycin's binding to three ribosomal sites may limit resistance from emerging.

A Clinical Laboratory and Standards Institute (CLSI) M23 type quality control (QC) study was performed to establish disk diffusion (DD) and agar dilution (AD) QC ranges for the *N. gonorrhoeae* ATCC 49226 strain to assist clinical laboratories in monitoring the *in vitro* activity of this compound during clinical trials and subsequent routine antimicrobial susceptibility testing.

MATERIALS AND METHODS

A total of nine laboratories were recruited to provide data for this QC investigation. For AD testing, three different manufacturers were used to supply GC agar base media lots: Becton Dickinson BBL (BD; Sparks, Maryland, USA), Hardy Criterion (Hardy Diagnostics; Santa Maria, California, USA) and Remel Oxoid (Hampshire, United Kingdom [UK]). Solithromycin was provided by Cempra Pharmaceuticals Inc (Chapel Hill, North Carolina, USA); ciprofloxacin was acquired from Sigma-Aldrich (St. Louis, Missouri, USA). Appropriate working concentrations were prepared to achieve the following range of test concentrations: 0.008 µg/mL to 0.5 µg/mL. Internal quality control testing was performed using ciprofloxacin, with a test concentration range of 0.0005 µg/mL to 0.015 µg/mL. All laboratories performed the testing over at least two days, with no more than five replicates being tested on one day. Each replicate represented an individually prepared inoculum suspension.

For DD testing, two lots of 15-µg solithromycin disks were manufactured by two different companies: MAST Group (Merseyside, United Kingdom UK) and BioRad (Marnes la Coquette, France). A single lot of comparator disks from BD was used as an internal control: ciprofloxacin 5-µg. Three different lots of GC agar, with 1% growth supplement, from two manufacturers were used for DD testing: Remel (Lenexa, Kansas, USA) and BD (two lots). Each laboratory site used two disk lots from two different manufacturers, generating two zone diameters (one with each disk lot) on three different media lots for 10 replicates, ultimately resulting in 60 determinations. Laboratories performed testing over at least three days with no more than four replicates tested on one day. GC agar plates were inoculated from a 0.5 McFarland suspension of *N. gonorrhoeae* (ATCC 49226) following standard operating procedures for DD, and two solithromycin disks and one ciprofloxacin disk were applied. Agar plates were incubated at 35°C; 5% CO_2 for 20-24 hours, after which zone diameters were manually determined.

RESULTS

- The proposed QC ranges for solithromycin for AD and DD testing against *N. gonorrhoeae* ATCC 49226 are summarized in **Table 1**.
- For AD testing, all (100.0%) reported MIC results would be in the proposed limits of 0.03 – 0.25 µg/mL for solithromycin. The "shoulder" at 0.06 µg/mL represents 80.5% of the modal 0.12 µg/mL MIC value. Therefore, a four $\log_2$ dilution range was proposed for the MIC QC range using the CLSI M23-A3 criteria (**Figure 1**). No significant skewing of results and modal MIC values was observed among media lots or laboratories; all of the observed solithromycin AD modal MIC values were within one doubling dilution of 0.12 µg/mL.
- Ciprofloxacin was used as an antimicrobial control agent, and all MIC values were within the CLSI published QC range (0.001-0.008 µg/mL), therefore providing a validated internal control for this study (**Table 2**).
- For DD testing, the zone diameters reported by the eight participating laboratories for solithromycin against the QC *N. gonorrhoeae* produced a 9 mm range (34-42 mm), which included 95.8% of all reported zone diameters (**Figures 2** and **3**). In addition, the Range Finder statistical program was applied to evaluate the ranges of zone diameter results. The Range Finder program suggested a slightly wider DD QC range (33-43 mm), which included 98.5% of reported zone diameters. No outliers were identified among the eight laboratories based on the median, geometric mean, and mode of the disk diameters.
- The control disks (ciprofloxacin) provided a valid internal control (240 zone diameters generated); and all but one zone diameter (239/240, 99.6%) recorded were within CLSI published QC guidelines for ciprofloxacin (48-58 mm).

Table 1. Quality control ranges of solithromycin disk diffusion (DD) and agar dilution (AD) testing against *N. gonorrhoeae* (ATCC 49226).

QC organism	DD zone diameters		AD MIC values	
	Proposed / approved range (mm)	% isolates tested in range	MIC range (µg/mL)	% isolates tested in range
N. gonorrhoeae (ATCC 49226)	34 – 42 **(33 – 43)***	95.8 (98.5)	0.03 – 0.25	100.0

* Range Finder calculations, subsequently approved by CLSI Subcommittee on Antimicrobial Susceptibility Testing in January 2015

Table 2. Results for ciprofloxacin DD and AD against *N. gonorrhoeae* (ATCC 49226) for internal control testing.

QC organism	DD zone diameters (mm)[a,*] [N=240 observations by 8 laboratories]			AD MIC values (µg/mL)[b] [N=80 observations by 8 laboratories]		
	Median	Geometric Mean	Range	Median	Geometric Mean	Range
N. gonorrhoeae (ATCC 49226)	54	53.8	47 – 58*	0.008	(n/a)	0.004 – 0.008

a. CLSI M23-A3 QC range for DD: 48 – 58 mm
b. CLSI M23-A3 QC range for AD: 0.001 – 0.008 µg/mL
* 239/240 (99.6%) of qualified DD testing results were within the published CLSI QC range, therefore providing a validated internal control

Figure 1. Solithromycin MIC distributions for *N. gonorrhoeae* (ATCC 49226) by agar media lots using AD testing.

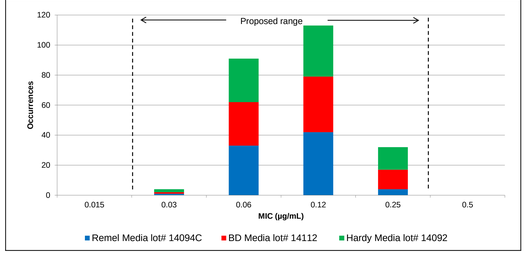


Figure 2. Solithromycin zone diameter distributions for *N. gonorrhoeae* (ATCC 49226) by agar media lots using DD testing.

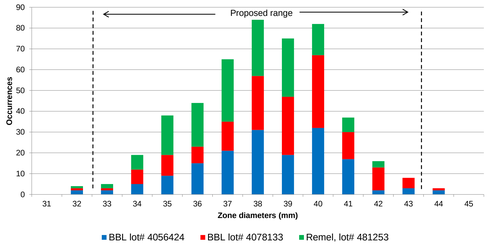
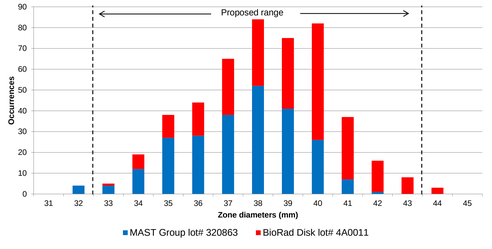


Figure 3. Solithromycin zone diameter distributions for *N. gonorrhoeae* (ATCC 49226) by disk lots using DD testing.



CONCLUSIONS

- The proposed QC ranges for solithromycin for AD and DD have generally good inter- and intra-laboratory reproducibility for the quality control strain, *N. gonorrhoeae* ATCC 49226.
- These proposed ranges were presented to the CLSI Antimicrobial Susceptibility Testing (AST) QC working group in January 2015. The AST subcommittee approved the Range Finder range for DD method (33-43 mm) which provided broader limits, and approved the AD method range of 0.03 – 0.25 µg/mL to be published in future CLSI M100 documents.
- These ranges can now be used to provide QC guidelines for laboratories performing antimicrobial susceptibility testing of solithromycin against *N. gonorrhoeae* in subsequent development and evaluations of this antimicrobial agent for broader clinical use for the treatment of infections caused by *N. gonorrhoeae*.

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