Determination of Disk Diffusion and MIC Quality Control Guidelines for Solithromycin, a Novel Fluoroketolide Antibacterial, against Neisseria gonorrhoeae

Stefan Riedela, James E. Rossb, David J. Farrell, Robert K. Flamm, Ronald N. Jonesb, c
The Johns Hopkins University, Baltimore, Maryland, USAa; JMI Laboratories, North Liberty, Iowa, USAa; Tufts University School of Medicine, Boston, Massachusetts, USAc

This solithromycin quality control study was performed to establish quality control (QC) ranges for the N. gonorrhoeae ATCC 49226 control strain for MIC agar dilution testing (AD) and zones by disk diffusion testing (DD). The following ranges were established: AD, 0.03 to 0.25 μg/ml, and DD, 33 to 43 mm. In January 2015, the CLSI Subcommittee on Antimicrobial Susceptibility Testing approved these ranges, which will be important when evaluating solithromycin against clinical isolates of N. gonorrhoeae.

In the past 2 decades, Neisseria gonorrhoeae has demonstrated a remarkable capacity to develop resistance to many of the antimicrobial agents commonly used for the treatment of gonorrhea (1). Solithromycin (formerly CEM-101) recently became the first fluoroketolide to enter clinical development. It has shown advantages in spectrum and potency over older macrolides against many Gram-positive and fastidious Gram-negative bacterial pathogens, including N. gonorrhoeae (2–5). A Clinical and Laboratory Standards Institute (CLSI) M23-style quality control (QC) study was performed to establish disk diffusion (DD) and agar dilution (AD) QC ranges for the N. gonorrhoeae ATCC 49226 control strain to assist clinical laboratories in monitoring the in vitro activity of this compound during clinical trial development and routine antimicrobial susceptibility testing (6–9).

Nine laboratories participated in this study to establish QC ranges (6). These laboratories were experienced microbiology facilities, and each used procedures in accordance with the CLSI guidelines for DD and AD methods (6–9). The following sites participated, and all performed DD and AD, unless otherwise noted: Summa Health Systems, Akron, OH, USA (G. Kallstrom); JMI Laboratories, North Liberty, IA, USA (R. N. Jones); Thermo Fisher Scientific, Cleveland, OH, USA (C. Knapp); Cleveland Clinic Foundation, Cleveland, OH, USA (G. Procop); University of Washington Medical Center, Seattle, WA, USA (S. Swanz); University of Rochester Medical Center, Rochester, NY, USA (D. Hardy; AD only); Wheaton Franciscan Laboratory, Wauwatosa, WI, USA (E. Munson; DD only); Johns Hopkins Bayview Medical Center, Baltimore, MD, USA (S. Riedel); and the University of Alberta Hospitals, Edmonton, Alberta, Canada (R. Rennie).

For AD testing, three different lots of GC agar manufactured by three different companies were used: Hardy Criterior (lot no. 14092), BD BBL (lot no. 14112), and Remel Oxoid (lot no. 14094 C). IsoVitaleX from BD (lot no. 4099231) was added to the GC agar base for growth enrichment. From a 1,000 μg/ml solithromycin stock solution (0.5 ml) (Cempra; lot no. GNS 10530), appropriate working concentrations were prepared to achieve a range of test concentrations of 0.008 to 0.5 μg/ml. The eight laboratories prepared their own agar dilution plates, in accordance with the standardized operating procedure for the preparation of agar plates, as provided by JMI and described in the CLSI document M07-A10 (8). For inoculum preparation, colonies of N. gonorrhoeae (ATCC strain 49226) from a chocolate agar plate (20 to 24 h of incubation) were suspended in Mueller-Hinton broth or saline to prepare a solution adjusted to a 0.5 McFarland standard density. The agar plates were inoculated with 1 to 2 μl of each suspension using an inoculum-replicating apparatus (e.g., Steers replicator) or equivalent device. Agar growth control plates (no antimicrobial agent added) were inoculated at the beginning and end of every test run to ensure that there was no contamination or significant antimicrobial carryover during the inoculation. All laboratories performed the testing over ≥2 days (day 1 agar, plate preparation; day 2, susceptibility testing), with no more than 5 replicates being tested on 1 day. Each replicate represented an individually prepared inoculum suspension. Experiments were performed by generating one MIC in three different medium lots for 10 replicates (30 determinants) per site for solithromycin at each participating laboratory sites, resulting in a total of 240 MIC values. The endpoints for determining the MIC by AD testing were interpreted by all participating laboratories as no visible growth on an agar plate for a specific antimicrobial concentration. Internal QC testing was performed using ciprofloxacin (lot no. BCB9941V; Sigma Aldrich) at a test concentration range of 0.0005 to 0.015 μg/ml. All ciprofloxacin MIC QC values were observed within the CLSI published QC range (0.001 to 0.008 μg/ml), therefore providing a validated internal control for this study (data not shown). To verify the accuracy of the prepared inocula, colony counts were performed by subculturing in a quantitative manner onto antimicrobial-free agar plates, resulting in an
average count of 1.2 × 10⁵ CFU/spot (range, 0.3 × 10⁵ to 1.8 × 10⁵ CFU/spot).

For DD testing, two different lots of 15-µg solithromycin disks were manufactured by two companies: Mast Group, Merseyside, United Kingdom (lot no. 320863) and Bio-Rad, Marnes la Coquette, France (lot no. 4A0011). A single lot of 5-µg ciprofloxacin disks from BD was used as an internal control (BD lot no. 3261282). Three different lots of GC agar with 1% growth supplement from 2 manufacturers were used for DD testing: Remel, Lenexa, KS, USA (lot no. 481253), and BD, Sparks, MD, USA (lot no. 4078133 and 4056424). Eight laboratories participated in DD testing; each laboratory used 2 disk lots from two different manufacturers, generating two zone diameters (one with each disk lot) on three different medium lots for 10 replicates, ultimately resulting in 60 determinations. The laboratories performed testing over ≈3 days, with no more than four replicates tested on 1 day. GC agar plates were inoculated from a 0.5 McFarland suspension of N. gonorrhoeae (strain ATCC 49226), in accordance with standard operating procedures for DD, as specified in CLSI M02-A12 (7), and two solithromycin disks and one ciprofloxacin disk were applied. The agar plates were incubated at 35°C with 5% CO₂ for 20 to 24 h, after which the zone diameters were manually determined.

The proposed QC ranges for solithromycin for AD and DD testing against N. gonorrhoeae (ATCC 49226) are summarized in Table 1. The solithromycin agar dilution MIC results obtained by the 8 laboratories are shown in Fig. 1. A bimodal distribution at 0.06 and 0.12 µg/ml was observed for the solithromycin MIC results against N. gonorrhoeae (ATCC 49226). The "shoulder" at 0.06 µg/ml represented 80.5% of the modal 0.12 µg/ml MIC value. Therefore, a 4-log₂ dilution range was proposed for the MIC QC range (0.03 to 0.25 µg/ml) using the CLSI M23-A3 criteria (6); all 240 reported results (100%) were within the proposed limits for solithromycin. No significant skewing of results or modal MIC values was observed among the medium lots. All of the observed solithromycin AD modal MIC values from each of the participating laboratories were within one doubling dilution of 0.12 µg/ml.

In addition to AD, disk diffusion testing was performed for solithromycin to establish QC ranges for N. gonorrhoeae ATCC 49226 (Table 1). When applying CLSI M23-A3 criteria (6), the zone diameters reported by the eight participating laboratories for solithromycin against the QC N. gonorrhoeae produced a 9-mm range (34 to 42 mm), which included 95.8% of all reported zone diameters. In addition, the Range Finder statistical program (10) was applied to evaluate the ranges of the MIC and zone diameter results (10). The Range Finder statistical program suggested a slightly wider DD QC range (33 to 43 mm), which included 98.5% of the reported zone diameters. No outliers were identified among the eight laboratories based on the median, geometric mean, and mode disk diameters (10). Minor and acceptable differences (≤1 mm) were observed between the range and median values of zone diameters observed with the two lots of solithromycin disks (Fig. 2). Twelve of 240 observations (5%) for Bio-Rad lot no. 4A0011 and 8/240 observations (3.33%) for Mast Group lot no. 320863 were outside the proposed QC range of 34 to 42 mm. Similarly, very little difference (≤2 mm) was observed between the range and median zone diameters occurring with the three agar medium lots (Fig. 3). These minor differences between the disk lots and agar medium lots were not statistically significant. 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 to 58 mm).

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

<table>
<thead>
<tr>
<th>QC organism</th>
<th>Disk diffusion zone diameters for CLSI/Range Finder</th>
<th>Agar dilution MIC values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoeae (ATCC 49226)</td>
<td>Proposed/Approved range (mm)</td>
<td>% isolates tested in range</td>
</tr>
<tr>
<td>34–42/33–43</td>
<td>95.8/98.5</td>
<td>0.03–0.25</td>
</tr>
</tbody>
</table>

* Range Finder calculations (10), if different from the CLSI M23-A3 ranges (6), were subsequently approved by the CLSI Subcommittee on Antimicrobial Susceptibility Testing in January 2015.
The proposed solithromycin QC ranges against *N. gonorrhoeae* (ATCC 49226) were presented to the CLSI Subcommittee on Antimicrobial Susceptibility Testing in January 2015 and subsequently approved for future publication in the CLSI M100-S25 document (9). The subcommittee elected to approve the Range Finder ranges for the DD method (33 to 43 mm), which provided broader limits (10). The results (Table 1) from this multilaboratory study now provide QC ranges for routine susceptibility testing when applying DD and AD methods, as defined by the CLSI (6–8), for this new fluoroketolide antimicrobial agent (2–5). These ranges can now be used to provide QC guidelines for laboratories performing antimicrobial susceptibility testing of solithromycin against *N. gonorrhoeae* for the subsequent development and evaluation of this antimicrobial agent for broader clinical use and the treatment of infections due to *N. gonorrhoeae*.

ACKNOWLEDGMENTS

This study was sponsored by a research grant from Cempra Pharmaceuticals, Inc., (Chapel Hill, NC, USA). J.E.R., D.J.F., R.K.F., and R.N.J. are employees of JMI Laboratories and receive grant funds to study solithromycin. S.R. declares no conflicts of interest.

We thank the nine contributing laboratories (personnel and directors) for their high-quality support of this complex protocol.

REFERENCES