Determination of Disk Diffusion and MIC Quality Control Guidelines for JNJ-Q2, a Novel Quinolone

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JNJ-Q2 is a novel fluorinated 4-quinolone in development for treatment of acute bacterial skin and skin structure infection and community-acquired bacterial pneumonia. This quality control (QC) study was performed to establish ranges for control strains: *Staphylococcus aureus* ATCC 29213 (0.004 to 0.015 μg/ml), *Enterococcus faecalis* ATCC 29212 (0.015 to 0.06 μg/ml), *Pseudomonas aeruginosa* ATCC 27853 (0.5 to 2 μg/ml and 17 to 23 mm), *Escherichia coli* ATCC 25922 (0.008 to 0.03 μg/ml and 30 to 36 mm), *Haemophilus influenzae* ATCC 49247 (0.002 to 0.015 μg/ml and 31 to 39 mm), and *S. aureus* ATCC 25923 (32 to 38 mm). These ranges will be crucial in evaluating JNJ-Q2 potency as it progresses through clinical trial development.

The fluoroquinolone class has been widely used to treat various human infections, including methicillin-resistant *Staphylococcus aureus* (MRSA). Rates of MRSA resistance to fluoroquinolones have risen >70% globally (1), but they have recently decreased in the United States (8). JNJ-Q2 is a novel fluorinated 4-quinolone that has demonstrated potent activity against Gram-negative and -positive pathogens, including MRSA and multidrug-resistant (MDR) *Streptococcus pneumoniae* (7, 9). It is currently being developed for the treatment of acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP). A Clinical Laboratory and Standards Institute (CLSI) M23 style quality control (QC) study was performed to establish disk diffusion and broth microdilution QC ranges for seven bacterial strains to assist clinical laboratories in monitoring the activity of this compound during ongoing clinical trials (2).

Eight laboratories (seven being required by CLSI M23-A3 guidelines) (5) were used in these two studies to establish a QC range. These laboratories were established microbiology facilities and each followed the CLSI procedures for disk diffusion (3) and broth microdilution (4) methods. The following sites participated: Massachusetts General Hospital, Boston, MA (M. J. Ferraro); Wheaton Franciscan Laboratory, Wauwatosa, WI (E. Munson); JMI Laboratories, North Liberty, IA (R. N. Jones); Trek Diagnostics, Cleveland, OH (C. Knapp); University of Alberta, Edmonton, Alberta, Canada (R. Rennie); University of Washington, Seattle, WA (S. Swanzey); University of Texas Medical Center, Houston, TX (A. Wanger); Robert Wood Johnson Medical School, New Brunswick, NJ (M. Weinstein; MIC study only); and the Cleveland Clinic Foundation, Cleveland, OH (G. Hall; disk diffusion study only).

Reference frozen-form broth microdilution panels were prepared by Trek Diagnostics according to good manufacturing practice (GMP) guidelines and shipped frozen to all participating sites. Panels contained four lots of cation-adjusted Mu-

### Table 1. Proposed quality control ranges of JNJ-Q2 disk diffusion and broth microdilution tests

<table>
<thead>
<tr>
<th>QC organism</th>
<th>Broth microdilution</th>
<th>Disk diffusion zone diam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed range (μg/ml)</td>
<td>% results in proposed range</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>0.015–0.06</td>
<td>100.0</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>0.004–0.015</td>
<td>100.0</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> ATCC 49619</td>
<td>0.004–0.015</td>
<td>100.0</td>
</tr>
<tr>
<td><em>H. influenzae</em> ATCC 49247</td>
<td>0.002–0.015</td>
<td>100.0</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>0.008–0.03</td>
<td>100.0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 27853</td>
<td>0.5–2</td>
<td>98.8</td>
</tr>
</tbody>
</table>

*NA, not available.

Range Finder results, if different, are in parentheses (10).
eller-Hinton broth (Oxoid, Hampshire, United Kingdom; BBL, Sparks, MD; and Difco [2 lots], Detroit, MI). Also, panels containing four lots of Haemophilus test medium (HTM) and four lots of Mueller-Hinton broth supplemented with 2 to 5% lysed horse blood were provided by the same vendor. Ciprofloxacin, moxifloxacin, and levofloxacin were utilized as control agents (5). For broth microdilution testing, each laboratory tested 10 replicates of S. aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Haemophilus influenzae ATCC 49247, and S. pneumoniae ATCC 49619. Colony counts were performed on drug-free agar media and resulted in the following average counts by QC organism: S. aureus ATCC 29213, 3.9 × 10⁵ CFU/ml; E. faecalis ATCC 29212, 2.5 × 10⁵ CFU/ml; P. aeruginosa ATCC 27853, 4.6 × 10⁵ CFU/ml; E. coli ATCC 25922, 3.7 × 10⁵ CFU/ml; H. influenzae ATCC 49247, 3.0 × 10⁵ CFU/ml; and S. pneumoniae ATCC 49619, 2.1 × 10⁵ CFU/ml.

For disk diffusion tests, two different lots of 5-μg JNJ-Q2 disks were manufactured by two companies: MAST Group, Merseyside, United Kingdom (lot 267710), and Bio-Rad, Hercules, CA (lot 0D0010). Single lots of comparator disks from BD (Franklin Lakes, NJ) were used: ciprofloxacin, 5 μg (lot 812187); levofloxacin, 5 μg (lot 813510); and moxifloxacin, 5 μg (lot 843804). Three manufacturers (Remel, Lenexa, KS; Hardy Diagnostics, Santa Maria, CA; and BBL) were used to produce lots of Mueller-Hinton agar (lots 886501, 09352 [Criterion powder lot 10131D for S. aureus testing only], 0049207), Haemophilus test medium (lots 891812, 10083, 0068366), and Mueller-Hinton agar with 5% sheep blood (lots 886502, 10097, 0082776).

The broth microdilution MIC results for JNJ-Q2 are summarized as proposed ranges in Table 1. All S. aureus ATCC 29213 MIC results were included in the proposed 0.004- to 0.015-μg/ml range. E. faecalis ATCC 29212 also had a proposed three log₂ dilution range (0.015 to 0.06 μg/ml) for JNJ-Q2 that included all results with 95% of these results falling at the modal MIC of 0.03 μg/ml. For P. aeruginosa ATCC 27853, 98.8% of all results fell within the proposed three doubling dilution range of 0.5 to 2 μg/ml. All E. coli ATCC 25922 and S. pneumoniae ATCC 49619 results were within the proposed ranges. In contrast, when testing H. influenzae ATCC 49247 and JNJ-Q2, a four log₂ dilution range was needed according to CLSI recommendations (5), due to a shoulder adjacent to the modal MIC (0.008 μg/ml) of 60% or greater (Fig. 1). The range of 0.002 to 0.015 μg/ml includes 100.0% of reported results.

All broth medium lots and laboratories failed to exhibit any skewing of results, and all modal values were within one MIC dilution step, regardless of the QC strain tested. Levofloxacin results (960 of 960 values; 100.0%) were within published QC ranges (6) for the MIC panel tested, providing a valid internal control for this portion of the study. The vast majority of the results for ciprofloxacin (637 of 640; 99.5%) and moxifloxacin (318 of 320; 99.4%) were also within CLSI published ranges (6).

Using CLSI document M23 (5) for establishing QC ranges, a disk diffusion study was also performed to propose zone diameter ranges for JNJ-Q2, and those results are also summarized in Table 1. The zone diameters reported by eight laboratories for S. aureus ATCC 25923 produced a range of 32 to 38 mm (7-mm range), which included 99.6% of these results (Fig. 2). P. aeruginosa ATCC 27853, E. coli ATCC 25922, H. influenzae ATCC 49247, and S. pneumoniae ATCC 49619 had proposed ranges with a span of 7 to 9 mm, and 95.2 to 100.0% of participant results were within these limits. The Range Finder method (10) suggested narrower ranges for two organisms (Table 1) and a wider range, 27 to 36 mm, for S. pneumoniae ATCC 49619. The range of 30 to 36 mm for E. coli ATCC 25922 included 96.5% of reported values, with all of the outlier diameters submitted by one laboratory (17 values). Examination of the raw data and consultation with the outlier laboratory participant could not determine a technical cause for these discordant values. These secondary statistical analyses (10) did not improve the validity of selected ranges.

The control disks (ciprofloxacin, levofloxacin, moxifloxacin) provided a valid internal control for this study, with 99.2 to 100.0% of participant zones within the CLSI’s published QC ranges (6). There was minimal variance in results between the two lots of JNJ-Q2 disks (no difference in median values) among six QC organisms tested. Agar medium lot differences were also minimal (medians, ≤2 mm).

These results (Table 1) from this multilaboratory study provide initial JNJ-Q2 QC ranges for routine susceptibility testing.
using disk diffusion and broth microdilution methods (3, 4), as this new fluoroquinolone progresses through phase 2 and 3 clinical trials for ABSSSI and CABP. These QC ranges were narrow and should function well as this potent fluoroquinolone (JNJ-Q2) moves onward in clinical development (2, 7, 9).

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REFERENCES


