D-1433

# Recommended Ulifloxacin (Prulifloxacin) MIC Quality Control Ranges for *Campylobacter jejuni* ATCC 33560 Using a CLSI Multi-Laboratory M23-A3 Study Design

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**ICAAC 2009**
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## AMENDED ABSTRACT

**Background:** Prulifloxacin, is a newer fluoroquinolone that is currently under development for infectious diarrhea. Its active metabolite, ulifloxacin, exhibits broad activity against enteric and non-enteric bacilli including enteropathogenic species. This study was performed to re-establish QC ranges for *C. jejuni* ATCC 33560 after discovering the previous MIC ranges were erroneously elevated (ICAAC 2006).

**Methods:** This broth microdilution QC study followed the M23-A3 (2008) guideline using 8 laboratories, 3 different manufacturers of cation-adjusted Mueller-Hinton broth with 2-5% lysed horse blood (4 lots) and used ciprofloxacin as the control agent. Two incubation conditions were utilized in accordance with CLSI M45-A for testing *C. jejuni*. Ten replicates were performed in four media lots at both 36ºC read at 48 h and 42ºC read at 24 h incubation in a microaerophilic environment.

**Results:** 4 of 8 sites enrolled in the study reported reduced or no growth of the *C. jejuni* strain at 42ºC in the MIC panel. One of those laboratories also reported no growth at 36ºC. The modal ulifloxacin MIC at 36ºC for 48 h was 0.06 µg/ml (65.7% of all results) and one $\log_2$ dilution step lower (0.03 µg/ml; 71.7%) at 42ºC for 24 h. All results for *C. jejuni* ATCC 33560 against ciprofloxacin were within published CLSI ranges.

| | Proposed ulifloxacin MIC QC ranges: | |
|---|---|---|
| QC organism | MIC range (µg/ml) | % in range |
| *C. jejuni* ATCC 33560 at 36ºC for 48 h (seven laboratories) | 0.03 – 0.12 | 100.0 |
| *C. jejuni* ATCC 33560 at 42ºC for 24 h (five laboratories) | 0.015 – 0.06 | 100.0 |

**Conclusions:** *C. jejuni* testing was problematic at incubation conditions of 42ºC read at 24 h, however, using results from 5 laboratories, a proposed range could be established for ulifloxacin (not ideal). Proposed MIC QC ranges for ulifloxacin (36ºC read at 48h) will help guide clinical or reference laboratories when testing clinical trial isolates and should facilitate the regulatory review process for this new compound.

## INTRODUCTION

Prulifloxacin is a newer fluoroquinolone that is currently under development for infectious diarrhea. Prulifloxacin is metabolized by esterases to produce the active metabolite, ulifloxacin. This antimicrobial agent has positive pharmacological properties including excellent pharmacodynamic target attainment, long-elimination half-life which allows for once-daily dosing, as well as excellent in-vitro potency. It exhibits broad activity against the Enterobacteriaceae and non-enteric bacilli including enteropathogenic species. Clinical trials have shown prulifloxacin's once-daily dosing is well tolerated.

The previous M23 quality control (QC) study in 2005 proposed QC ranges for *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Campylobacter jejuni* ATCC 33560. Further testing at JMI Laboratories discovered that panels produced by a GMP vendor for *C. jejuni* were manufactured incorrectly which created elevated ulifloxacin MIC values (>10-fold). Upon recognition of this error, a decision was made to repeat the *C. jejuni* portion of the QC study. This multicenter study using Clinical and Laboratory Standards Institute (CLSI) methods adhering to the M23-A3 study design was performed to re-establish QC ranges for *C. jejuni* ATCC 33560.

## MATERIALS AND METHODS

Following the M23-A3 guidelines for a broth microdilution QC study, a total of eight laboratories were recruited to provide sufficient data for this repeated investigation. A total of four cation-adjusted Mueller-Hinton broth media lots with 2-5% lysed horse blood from 3 different manufacturers (Difco Laboratories (Detroit, MI), Becton Dickinson (BD Sparks, MD), and Oxoid (Hampshire, United Kingdom) were used in the production of these panels. Ulifloxacin was provided by Optimer Pharmaceuticals, Inc. (San Diego, CA); ciprofloxacin was acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified GMP source, TREK Diagnostics (Cleveland, OH).

Concurrent testing of ciprofloxacin was utilized as a control agent. Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates. The average colony counts among the participating centers was 2.4 X $10^5$ CFU/ml and ranged from 0.7 X $10^5$ CFU/ml to 7.1 X $10^5$ CFU/ml.

Two incubation conditions were utilized in accordance with CLSI M45-A for testing *C. jejuni*. Ten replicates were performed in 4 broth media lots at both 36ºC read at 48 hours and 42ºC read at 24 hours incubation in a microaerophilic environment.

## RESULTS

- Four of the eight laboratories enrolled in the study reported reduced or no growth of the *C. jejuni* QC strain at 42ºC in the MIC panel. One of those laboratories also reported no growth of the strain at 36ºC.
- The ulifloxacin MIC results from seven qualifying laboratories for *C. jejuni* ATCC 33560 incubated at 36ºC for 48 hours are shown in Tables 1 and 2 as well as Figure 1. Using M23 criteria to establish MIC QC ranges, 100.0% of all reported results would be in the proposed limits of 0.03 – 0.12 µg/ml (mode ± one $\log_2$ dilution step).
- Using results from only 5 laboratories (184 values), Tables 1 and 2 and Figure 2 shows ulifloxacin MIC results with *C. jejuni* ATCC 33560 incubated at the alternate temperature of 42ºC for only 24 hours. The proposed range of 0.015 – 0.06 µg/ml includes 100.0% of values reported and one $\log_2$ dilution step lower than the range for the 36ºC incubation temperature (Figure 1).
- Concurrent testing of ciprofloxacin was utilized as a control agent. Tables 1 and 2 as well as Figures 3 and 4 show *C. jejuni* ATCC 33560 MIC results with all QC results within the CLSI published range for ciprofloxacin (both incubation conditions).
- No significant variation was noted among media lots or laboratories for either incubation condition. Only one laboratory at each incubation condition had a differing modal MIC value.
- The alternative "range finder" statistical method suggested the same practical three $\log_2$ dilution range for ulifloxacin.

**Table 2**. Comparison of the ulifloxacin and ciprofloxacin MIC distributions among four lots of Mueller-Hinton broth when testing *C. jejuni* ATCC 33960.

| Incubation degrees[a]/ time period[b] | MIC (µg/ml) | Occurrences by lot[c]: A | B | C | D | Total |
|---|---|---|---|---|---|---|
| Ulifloxacin 36/48 | 0.03 | 10 | 7 | 5 | 1 | 23 |
| | 0.06 | 51 | 45 | 44 | 44 | 184 |
| | 0.12 | 9 | 18 | 21 | 25 | 73 |
| | Total | 70 | 70 | 70 | 70 | |
| | Mode | 0.06 | 0.06 | 0.06 | 0.06 | |
| | Range | 3 | 3 | 3 | 3 | |
| Ulifloxacin 42/24 | 0.015 | 2 | 2 | | | 4 |
| | 0.03 | 35 | 32 | 34 | 31 | 132 |
| | 0.06 | 9 | 12 | 12 | 15 | 48 |
| | Total | 46 | 46 | 46 | 46 | |
| | Mode | 0.03 | 0.03 | 0.03 | 0.03 | |
| | Range | 3 | 3 | 2 | 2 | |
| Ciprofloxacin 36/48 | 0.06 | 4 | 3 | 3 | 3 | 13 |
| | 0.12 | 42 | 43 | 50 | 47 | 182 |
| | 0.25 | 24 | 24 | 17 | 20 | 85 |
| | Total | 70 | 70 | 70 | 70 | |
| | Mode | 0.12 | 0.12 | 0.12 | 0.12 | |
| | Range | 3 | 3 | 3 | 3 | |
| Ciprofloxacin 42/24 | 0.03 | 1 | 1 | | | 2 |
| | 0.06 | 32 | 33 | 31 | 31 | 127 |
| | 0.12 | 13 | 12 | 15 | 15 | 55 |
| | Total | 46 | 46 | 46 | 46 | |
| | Mode | 0.06 | 0.06 | 0.06 | 0.06 | |
| | Range | 3 | 3 | 2 | 2 | |

a. Degrees Celsius.
b. Time period in hours for incubation.
c. Media lots included BD Lot #7143673; Difco Lot #7263432; Difco Lot #6135087; Oxoid Lot #332998.

**Table 1**. Inter and intra-laboratory comparisons of ulifloxacin and ciprofloxacin MIC results when testing *C. jejuni* ATCC 33560 for an eight laboratory protocol conforming to the study design guidelines found in CLSI M23-A3 (2008).

| Incubation degrees[a]/ time period[b] | MIC (µg/ml) | Laboratory code (occurrences): A | B | C | D | E | F | G | H | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Ulifloxacin 36/48 | 0.03 | | | | 8 | | 1 | 14 | NG[c] | 23[d] |
| | 0.06 | 27 | 19 | 22 | 32 | 20 | 39 | 25 | NG | 184[d] |
| | 0.12 | 13 | 21 | 18 | | 20 | | 1 | NG | 73[d] |
| | Total | 40 | 40 | 40 | 40 | 40 | 40 | 40 | | 280 |
| | Mode | 0.06 | 0.12 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | | 0.06 |
| | Range | 2 | 2 | 2 | 2 | 2 | 2 | 3 | | 3 |
| Ulifloxacin 42/24 | 0.015 | | NG | NG | 1 | 2 | | 1 | NG | 4[d] |
| | 0.03 | 10 | NG | NG | 15 | 38 | 36 | 33 | NG | 132[d] |
| | 0.06 | 30 | NG | NG | 8 | | 4 | 6 | NG | 48[d] |
| | Total | 40 | | | 23 | 40 | 40 | 40 | | 184 |
| | Mode | 0.06 | | | 0.03 | 0.03 | 0.03 | 0.03 | | 0.03 |
| | Range | 2 | | | 3 | 2 | 2 | 3 | | 3 |
| Ciprofloxacin 36/48 | 0.06 | | | | | | | 13 | NG | 13[d] |
| | 0.12 | 40 | 15 | 15 | 25 | 20 | 40 | 27 | NG | 182[d] |
| | 0.25 | | 25 | 25 | 15 | 20 | | | NG | 85[d] |
| | Total | 40 | 40 | 40 | 40 | 40 | 40 | 40 | | 280 |
| | Mode | 0.12 | 0.25 | 0.25 | 0.12 | 0.12 | 0.12 | 0.12 | | 0.12 |
| | Range | 1 | 2 | 2 | 2 | 2 | 1 | 2 | | 3 |
| Ciprofloxacin 42/24 | 0.03 | | NG | NG | | 2 | | | NG | 2[d] |
| | 0.06 | 6 | NG | NG | 15 | 36 | 32 | 38 | NG | 127[d] |
| | 0.12 | 34 | NG | NG | 9 | 2 | 8 | 2 | NG | 55[d] |
| | Total | 40 | | | 24 | 40 | 40 | 40 | | 184 |
| | Mode | 0.12 | | | 0.06 | 0.06 | 0.06 | 0.06 | | 0.06 |
| | Range | 2 | | | 2 | 3 | 2 | 2 | | 3 |

a. Degrees Celsius.
b. Time period in hours.
c. NG = no growth.
d. 100.0% of qualified results were within proposed ulifloxacin QC range (0.03-0.12 µg/ml).

**Figure 1**. Ulifloxacin MIC distribution for *C. jejuni* ATCC 33560 incubated at 36ºC for 48 hours.

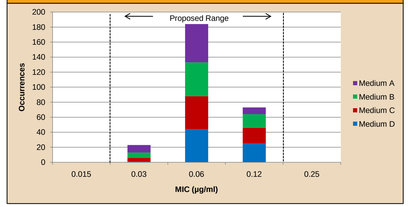



**Figure 2**. Ulifloxacin MIC distribution for *C. jejuni* ATCC 33560 incubated at 42ºC for 24 hours.

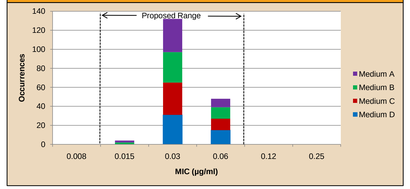



**Figure 3**. Ciprofloxacin MIC distribution for *C. jejuni* ATCC 33560 incubated at 36ºC for 48 hours.

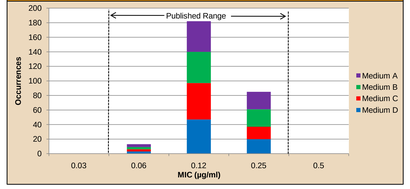
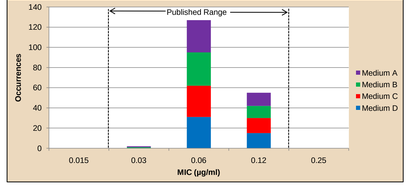



**Figure 4**. Ciprofloxacin MIC distribution for *C. jejuni* ATCC 33560 incubated at 42ºC for 24 hours.




## CONCLUSIONS

- The proposed QC ranges provided in this report show that laboratories can accurately test ulifloxacin (active component of prulifloxacin) with excellent inter- and intra-laboratory reproducibility for *C. jejuni* ATCC 33560.
- The following chart lists the recommended or proposed QC ranges for ulifloxacin under both incubation conditions using an ATCC *C. jejuni* QC strain:

| | Proposed ulifloxacin MIC QC ranges: | |
|---|---|---|
| QC organism | MIC range (µg/ml) | % in range |
| *C. jejuni* ATCC 33560 at 36ºC for 48 hours (seven laboratories) | 0.03 – 0.12* | 100.0 |
| *C. jejuni* ATCC 33560 at 42ºC for 24 hours (five laboratories) | 0.015 – 0.06 | 100.0 |

*Only one testing condition range is suggested

## ACKNOWLEDGEMENTS

This study was funded by a research grant from Optimer Pharmaceuticals.

Participating laboratories and principal investigators utilized for the determination of ulifloxacin (prulifloxacin) QC ranges included R.N. Jones (JMI Laboratories), C. Knapp (TREK Diagnostics), R. Rennie (University of Alberta), S. Mirrett (Duke University Medical Center), M.J. Ferraro (Massachusetts General Hospital), S. Swanzy, (University of Washington Medical Center), E. Munson (Wheaton Franciscan Laboratory) and M. Weinstein (Robert Wood Johnson Medical School).

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