

# Multicenter Evaluation of Ciprofloxacin MIC Results for Gram Negative Bacteria Using MicroScan Dried Gram Negative MIC Panels

A. Harrington<sup>1</sup>, S. DesJarlais<sup>1</sup>, J. A. Hindler<sup>2</sup>, S. A. Miller<sup>2</sup>, M. Traczewski<sup>3</sup>, D. Beasley<sup>3</sup>, F. Xu<sup>4</sup>, R. K. Brookman<sup>5</sup>, C. J. Hastey<sup>5</sup>, A. M. Chipman<sup>5</sup>, D. Roe-Carpenter<sup>5</sup>

<sup>1</sup>Loyola University Medical Center, Maywood, IL, <sup>2</sup>UCLA David Geffen School of Medicine, Los Angeles, CA, <sup>3</sup>Clinical Microbiology Institute, Wilsonville, OR, <sup>4</sup>California Department of Public Health, and <sup>5</sup>Beckman Coulter, West Sacramento, CA

## ABSTRACT

**Background:** A multicenter study was performed to evaluate the accuracy of an extended dilution series of ciprofloxacin on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels. Ciprofloxacin (FDA) will be identified or labeled as ciprofloxacin-S (Cp-S).

**Material/Methods:** For challenge, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 74 *Salmonella enterica* serovar Typhi (S. Typhi) isolates were tested at each of three sites (for a total of 222 replicates) using the turbidity and Prompt™ methods of inoculation. For reproducibility, a subset of 11 organisms was tested on MSDGN panels at all three sites. MSDGN panels were incubated at 35 ± 2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2°C and read visually. All frozen reference panels were read at 16-18 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Salmonella* Typhi ≤ 0.06 S, 0.12-0.5 I, and ≥ 1 R.

**Results:** When compared to frozen reference panel results, essential and categorical agreements for all isolates tested in Challenge are as follows:

| Read Method | Essential Agreement % |                 | Categorical Agreement % |                   | Very Major Errors % |               | Major Errors % |               | Minor Errors %  |                 |
|-------------|-----------------------|-----------------|-------------------------|-------------------|---------------------|---------------|----------------|---------------|-----------------|-----------------|
|             | T                     | P               | T                       | P                 | T                   | P             | T              | P             | T               | P               |
| Visually    | 100<br>(22/222)       | 100<br>(22/222) | 92.8<br>(209/222)       | 93.2<br>(207/222) | 0.0<br>(0/73)       | 0.0<br>(0/73) | 0.0<br>(0/54)  | 0.0<br>(0/54) | 7.2<br>(16/222) | 6.8<br>(15/222) |
| WalkAway    | 99.5<br>(21/222)      | 100<br>(22/222) | 94.1<br>(209/222)       | 93.7<br>(208/222) | 0.0<br>(0/73)       | 0.0<br>(0/73) | 0.0<br>(0/54)  | 0.0<br>(0/54) | 5.9<br>(13/222) | 6.3<br>(14/222) |
| autoSCAN-4  | 100<br>(22/222)       | 100<br>(22/222) | 95.0<br>(211/222)       | 95.5<br>(212/222) | 0.0<br>(0/73)       | 0.0<br>(0/73) | 0.0<br>(0/54)  | 0.0<br>(0/54) | 5.0<br>(11/222) | 4.5<br>(10/222) |

T = Turbidity inoculation method, P = Prompt inoculation method

Reproducibility among the three sites were greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.

**Conclusions:** This multicenter study showed that ciprofloxacin (identified as ciprofloxacin-S) MIC results for *Salmonella enterica* serovar Typhi obtained with the MSDGN panel with an extended dilution series correlate well with MICs obtained using frozen reference panels.

## INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with ciprofloxacin using *Salmonella* Typhi isolates with FDA interpretive breakpoints.

## METHODS

**Study Design:** MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel at three sites using both the turbidity and Prompt inoculation methods. A total of 74 *Salmonella enterica* serovar Typhi clinical isolates were tested at each of three sites.

**Quality Control Expected Results**  
*Escherichia coli* ATCC 25922: 0.004-0.016 µg/ml  
*Pseudomonas aeruginosa* ATCC 27853: 0.25-1 µg/ml

## METHODS (Continued)

### Panels

\*Frozen reference and MSDGN MIC panels contained two-fold doubling dilutions of ciprofloxacin 0.004-8 µg/ml in cation-adjusted Mueller-Hinton broth.

\*Reference panels were prepared and frozen following CLSI recommendations.

### Reproducibility

\*Reproducibility organisms with known results on-scale for ciprofloxacin were tested in triplicate (for each inoculation) on the MSDGN MIC panels and singly on the frozen reference panel on three different days at each site.

\*MSDGN MIC panels were tested using both the turbidity and Prompt inoculation methods and read on the WalkAway system, autoSCAN-4 instrument, and manually.

### Quality Control

\*Quality control (QC) testing was performed daily using ATCC 25922 *Escherichia coli*, ATCC 27853 *Pseudomonas aeruginosa*, using CLSI and FDA/CLSI QC ranges.

### Panel Inoculation, Incubation, and Reading

\*All isolates were subcultured into trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 35-37°C prior to testing. Isolates from frozen stocks were subcultured twice before testing.

\*Inoculum suspensions for each strain were prepared with the direct standardization (turbidity standard) method for MSDGN MIC and frozen reference panels. MSDGN MIC panels were also inoculated using the Prompt inoculation method.

\*Following inoculation, MSDGN MIC panels were incubated at 35 ± 2°C in the WalkAway system for 18 ± 2 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

### Data Analysis

\*Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference result MIC.

\*Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, I, and R) agree using FDA breakpoints for *Salmonella* Typhi (Table 1).

**Table 1. Ciprofloxacin FDA Interpretive Breakpoints (µg/ml)**

| Organism Group          | S      | I          | R   |
|-------------------------|--------|------------|-----|
| <i>Salmonella</i> Typhi | ≤ 0.06 | 0.12 – 0.5 | ≥ 1 |

\*Major Errors = Frozen reference MIC is S and MSDGN panel MIC is R; calculated for susceptible strains only.

$$\% \text{ Major Errors} = \frac{\text{No. Major Errors}}{\text{Total No. S Isolates tested}} \times 100$$

\*Very Major Errors = Frozen reference is R and MSDGN panel MIC is S; calculated for resistant strains only.

$$\% \text{ Very Major Errors} = \frac{\text{No. Very Major Errors}}{\text{Total No. R Isolates tested}} \times 100$$

\*Minor Errors = Frozen reference is S or R when MSDGN panel MIC is I or MSDGN panel MIC is S or R when frozen reference is I; calculated for all isolates tested.

$$\% \text{ Minor Errors} = \frac{\text{No. Minor Errors}}{\text{Total No. Isolates tested}} \times 100$$

## RESULTS

### Challenge (Triplicate data: Table 2 and 3)

\*A total of 74 *Salmonella* Typhi clinical isolates were tested at each of three clinical trial sites, for a total of 222 replicates. MSDGN panels were inoculated using the turbidity inoculation method.

\*Essential Agreement for clinical isolates between MSDGN panel and frozen reference panel was 100% (222/222) for manual read method, 99.5% (221/222) for WalkAway System, 100% (222/222) for autoSCAN-4 instrument using the turbidity inoculation method.

\*Categorical Agreement for clinical isolates between MSDGN panel and frozen reference panel was 92.8% (206/222) for manual read method, 94.1% (209/222) for WalkAway System, 95.0% (211/222) for autoSCAN-4 instrument using the turbidity inoculation method.

**Table 2. *Salmonella* Typhi Challenge – Turbidity Inoculation Method**

| Read Method | Essential Agreement |      | Categorical Agreement |      | Minor Errors |     | Major Errors |     | Very Major Errors |     |
|-------------|---------------------|------|-----------------------|------|--------------|-----|--------------|-----|-------------------|-----|
|             | No.                 | %    | No.                   | %    | No.          | %   | No.          | %   | No.               | %   |
| Manual      | 222/222             | 100  | 206/222               | 92.8 | 16/222       | 7.2 | 0/54         | 0.0 | 0/73              | 0.0 |
| WalkAway    | 221/222             | 99.5 | 209/222               | 94.1 | 13/222       | 5.9 | 0/54         | 0.0 | 0/73              | 0.0 |
| autoSCAN-4  | 222/222             | 100  | 211/222               | 95.0 | 11/222       | 5.0 | 0/54         | 0.0 | 0/73              | 0.0 |

\*A total of 74 *Salmonella* Typhi clinical isolates were tested at each of 3 clinical trial sites, for a total of 222 replicates. MSDGN panels were inoculated using the Prompt inoculation method.

\*Essential Agreement for clinical isolates between MSDGN panel and frozen reference panel was 100% (222/222) for manual read method, 100% (222/222) for WalkAway System, 100% (222/222) for autoSCAN-4 instrument using the Prompt inoculation method.

\*Categorical Agreement for clinical isolates between MSDGN panel and frozen reference panel was 93.2% (207/222) for manual read method, 93.7% (208/222) for WalkAway System, 95.5% (212/222) for autoSCAN-4 instrument using the Prompt inoculation method.

**Table 3. *Salmonella* Typhi Challenge – Prompt Inoculation Method**

| Read Method | Essential Agreement |     | Categorical Agreement |      | Minor Errors |     | Major Errors |     | Very Major Errors |     |
|-------------|---------------------|-----|-----------------------|------|--------------|-----|--------------|-----|-------------------|-----|
|             | No.                 | %   | No.                   | %    | No.          | %   | No.          | %   | No.               | %   |
| Manual      | 222/222             | 100 | 207/222               | 93.2 | 15/222       | 6.8 | 0/54         | 0.0 | 0/73              | 0.0 |
| WalkAway    | 222/222             | 100 | 208/222               | 93.7 | 14/222       | 6.3 | 0/54         | 0.0 | 0/73              | 0.0 |
| autoSCAN-4  | 222/222             | 100 | 212/222               | 95.5 | 10/222       | 4.5 | 0/54         | 0.0 | 0/73              | 0.0 |

### Challenge (Single replicate data: Table 4 & 5)

\*A total of 74 *Salmonella enterica* serovar Typhi (S. Typhi) stock isolates were tested at each of 3 sites. Site results were randomized to produce single replicate data using the turbidity inoculation method.

\*Essential Agreement for S. Typhi between MSDGN panel and frozen reference panel was 100% (74/74) for manual read method, 100% (74/74) for WalkAway System, 100% (74/74) for autoSCAN-4 instrument using the turbidity inoculation method.

\*Categorical Agreement for S. Typhi was 95.9% (71/74) for manual read method, 95.9% (71/74) for WalkAway System, 95.9% (71/74) for autoSCAN-4 instrument using the turbidity inoculation method.

**Table 4. *Salmonella* Typhi Challenge – Turbidity Inoculation Method**

| Read Method | Essential Agreement |     | Categorical Agreement |      | Minor Errors |     | Major Errors |     | Very Major Errors |     |
|-------------|---------------------|-----|-----------------------|------|--------------|-----|--------------|-----|-------------------|-----|
|             | No.                 | %   | No.                   | %    | No.          | %   | No.          | %   | No.               | %   |
| Manual      | 74/74               | 100 | 71/74                 | 95.9 | 3/74         | 4.1 | 0/18         | 0.0 | 0/26              | 0.0 |
| WalkAway    | 74/74               | 100 | 71/74                 | 95.9 | 3/74         | 4.1 | 0/18         | 0.0 | 0/26              | 0.0 |
| autoSCAN-4  | 74/74               | 100 | 71/74                 | 95.9 | 3/74         | 4.1 | 0/18         | 0.0 | 0/26              | 0.0 |

## RESULTS (Continued)

\*A total of 74 *Salmonella enterica* serovar Typhi (S. Typhi) stock isolates were tested at each of the three sites. Site results were randomized to produce single replicate data using the Prompt inoculation method.

**Table 5. *Salmonella* Typhi Challenge – Prompt Inoculation Method**

| Read Method | Essential Agreement |     | Categorical Agreement |      | Minor Errors |     | Major Errors |     | Very Major Errors |     |
|-------------|---------------------|-----|-----------------------|------|--------------|-----|--------------|-----|-------------------|-----|
|             | No.                 | %   | No.                   | %    | No.          | %   | No.          | %   | No.               | %   |
| Manual      | 74/74               | 100 | 70/74                 | 94.6 | 4/74         | 5.4 | 0/18         | 0.0 | 0/26              | 0.0 |
| WalkAway    | 74/74               | 100 | 70/74                 | 94.6 | 4/74         | 5.4 | 0/18         | 0.0 | 0/26              | 0.0 |
| autoSCAN-4  | 74/74               | 100 | 72/74                 | 97.3 | 2/74         | 2.7 | 0/18         | 0.0 | 0/26              | 0.0 |

\*Essential Agreement for S. Typhi between MSDGN panel and frozen reference panel was 100% (74/74) for manual read method, 100% (74/74) for WalkAway System, 100% (74/74) for autoSCAN-4 instrument using the Prompt inoculation method.

\*Categorical Agreement for S. Typhi was 94.6% (70/74) for manual read method, 94.6% (70/74) for WalkAway System, 97.3% (72/74) for autoSCAN-4 instrument using the Prompt inoculation method.

### Reproducibility (Table 6)

\*Overall agreement (within ± two-fold dilution) between all sites for the reproducibility phase was ≥95% for all combinations for best-case. Worst-case scenarios were <95% due to one isolate generating off-scale results (>8). All frozen reference results generated on each day of reproducibility testing were in agreement at >8 for that isolate. The remaining isolates were on-scale and met all acceptance criteria.

**Table 6. Reproducibility Testing with Ciprofloxacin-S Worst Case – All Sites Combined with All Instrument Reads of MDGN Panel**

| Read Method | Inoculation Method | No. (%) Agreement All Sites Combined |
|-------------|--------------------|--------------------------------------|
| Manual      | Turbidity          | 271/297 (91.2)                       |
| WalkAway    |                    | 269/297 (90.6)                       |
| autoSCAN-4  |                    | 270/297 (90.9)                       |
| Manual      | Prompt             | 270/297 (90.9)                       |
| WalkAway    |                    | 270/297 (90.9)                       |
| autoSCAN-4  |                    | 270/297 (90.9)                       |

### Quality Control (Table 7)

\*Overall QC results for the frozen reference panel were 100% in range (189/189) for *E. coli* and *P. aeruginosa*.

| Organism                        | QC Range (µg/mL) | Percent (%) In Range |                  |                  |                  |                 |                  |
|---------------------------------|------------------|----------------------|------------------|------------------|------------------|-----------------|------------------|
|                                 |                  | Manual               |                  | WalkAway         |                  | autoSCAN-4      |                  |
|                                 |                  | Turbidity            | Prompt           | Turbidity        | Prompt           | Turbidity       | Prompt           |
| <i>E. coli</i> ATCC 25922       | ≤ 0.004-0.015    | 188/189<br>99.4%     | 188/189<br>99.4% | 188/189<br>99.4% | 185/186<br>98.9% | 188/188<br>100% | 187/188<br>99.4% |
| <i>P. aeruginosa</i> ATCC 27853 | 0.25-1           | 189/189<br>100%      | 189/189<br>100%  | 189/189<br>100%  | 185/185<br>100%  | 187/187<br>100% | 188/189<br>100%  |

## CONCLUSION

There is a correlation between the MIC results obtained using MicroScan Dried Gram Negative panel and MICs obtained using a CLSI broth microdilution frozen reference panel for susceptibility testing of an extended formulation of ciprofloxacin-S (Cp-S) and *Salmonella enterica* serovar Typhi in a multicenter study with FDA interpretive criteria.

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