# Innate Errors of Quantitative Streaking Methodologies

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## ABSTRACT

**The objective of this study was to assess the variability of quantitative streaking between and within groups of laboratory professionals and an automated spiral plater. In today's clinical laboratories, advances in technology present an ever-changing landscape that mandates adaptations and the microbiology lab is no exception. Despite these advancements, one of the most quintessential manual techniques employed by laboratory professionals and taught to clinical laboratory science and technician students is quantitative streaking of bacterial cultures. Although few, there are studies detailing the accuracy of a 0.001 mL calibrated loop, perhaps the most common tool for quantitative streaking; however, there has been a lack of work addressing the variability associated with laboratory personnel's individual techniques and inherent variability. Our study analyzed the number of bacterial colony forming units (CFU)/mL that resulted from sequential plating by our control groups and automatic spiral plater from a common sample. The sample was a dilution of bacteria in saline from an initial 0.5 McFarland standard (approximation of $1.5x10^8$ CFU/mL). Preliminary data indicates that in most instances there were significant differences seen (via ANOVA and Tukey post hoc tests; $p<0.05$) within the test groups and considerable variations within each individual's plating results (measured by coefficient of variation) for both the gram-positive and gram-negative organism dilutions tested, *Staphylococcus aureus* and *Escherichia coli*, respectively. While this result is not unexpected, our work also shows that there are manual streaking procedural changes that more closely mimic the results obtained by our automated plating, circumventing potential lab budgetary constraints with purchasing automated platers. Collectively, our data demonstrates that manual quantitative streaking protocols are an area of the clinical microbiology lab that should be regularly assessed for quality control to ensure accuracy and reproducibility between laboratory professionals.**

## METHODS

- **Dilutions were made from 0.5 McFarland standards that gave an ideal colony number for enumeration, typically 1:1,000 or 1:10,000.**
- **The dilutions were then quantitatively streaked (Figure 1B) by randomly selected laboratory professionals, using a 1µl inoculation loop, representing a range of experience from student-level to 20+ years in a clinical microbiology laboratory. Plates were also streaked using the Autoplater 4000 from Spiral Biotech. For procedure comparisons, manual inoculation by a 1µl inoculation loop (Streak 1) was followed by spreading/streaking by an inoculation needle (Streak 2)-See Figure 1B.**
- **All plates were streaked on Trypticase Soy Agar w/5% Sheep Blood.**
- **Plates were incubated at 35°C for 18-24 hours in room air.**
- **Post-incubation, colony counts were determined for each plate and reported as colony forming units/milliliter (CFU/mL).**
- **Statistical analysis was performed using GraphPad Prism software.**

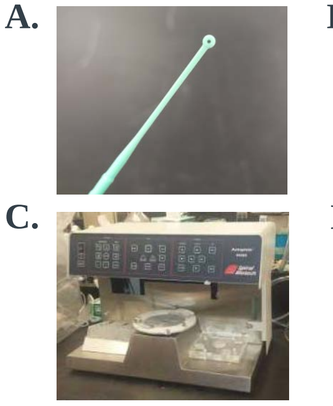
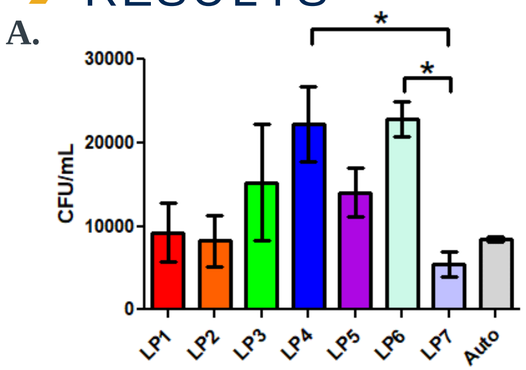



**Figure 1. Manual and automatic streaking methods. (A.)** Standard 1µl inoculation loop. **(B.)** Manual quantitative streaking pattern used. **(C.)** Autoplate 4000 by Spiral Biotech **(D.)** Grid pattern used for colony enumeration from the spiral plater.

## RESULTS




B.

Coefficient of Variation

| LP1 | LP2 | LP3 | LP4 | LP5 | LP6 | LP7 | Auto |
|---|---|---|---|---|---|---|---|
| 85.38% | 84.23% | 102.70% | 45.65% | 46.57% | 20.19% | 60.86% | **7.76%** |

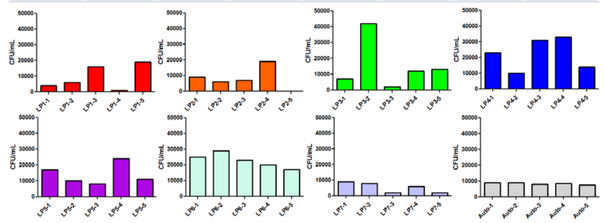
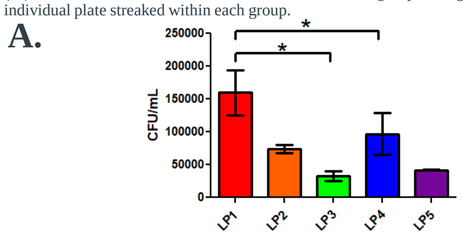


**Figure 2. Quantitative streaking of a 1:10,000 dilution of a 0.5 McFarland standard of *Staphylococcus aureus* in sterile saline. (A.)** Plate group comparison between seven laboratory professionals and the spiral plater. One-way ANOVA $p\leq0.01$**. Asterisks indicate Tukey post-test demonstrating statistical significance between groups $p\leq0.05$* **(B.)** Coefficient of variation was calculated for each test group. Bar graphs represent each individual plate streaked within each group.




B.

Coefficient of Variation

| LP1 | LP2 | LP3 | LP4 | LP5 |
|---|---|---|---|---|
| 37.35% | 14.95% | 36.90% | 56.81% | 4.76% |

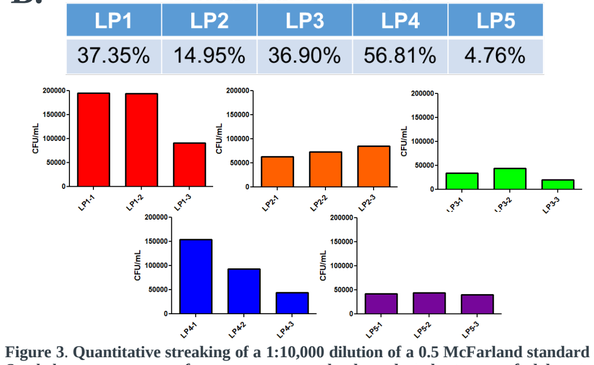
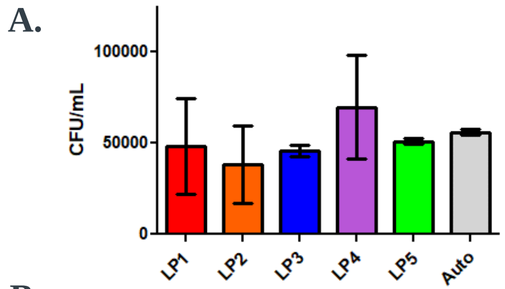


**Figure 3. Quantitative streaking of a 1:10,000 dilution of a 0.5 McFarland standard of *Staphylococcus aureus* from a new randomly selected group of laboratory professionals. (A.)** Plate group comparison between five laboratory professionals. One-way ANOVA $p\leq0.05$*. Asterisks indicate Tukey post-test demonstrating statistical significance between groups $p\leq0.05$* **(B.)** Coefficient of variation was calculated for each test group. Bar graphs represent each individual plate streaked within each group.




B.

Coefficient of Variation

| LP1 | LP2 | LP3 | LP4 | LP5 | Auto |
|---|---|---|---|---|---|
| 94.12% | 96.53% | 12.45% | 70.91% | 5.19% | 6.43% |

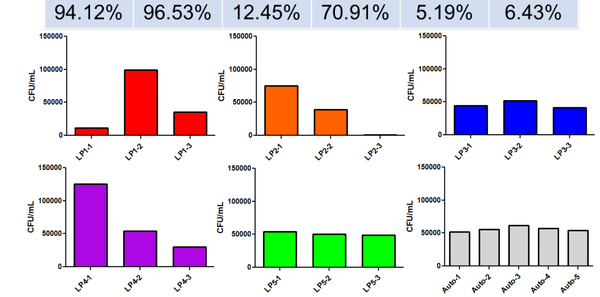
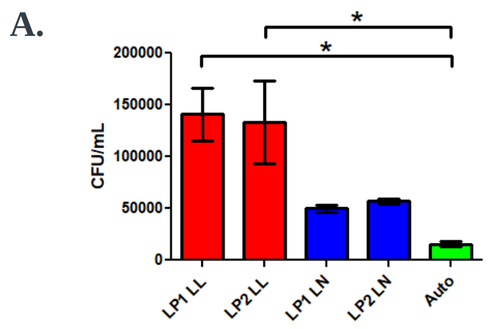


**Figure 4. Quantitative streaking of a 1:1,000 dilution of a 0.5 McFarland standard of *Escherichia coli* in sterile saline. (A.)** Plate group comparison between five laboratory professionals and the spiral plater. No statistical significance was found by one-way ANOVA. **(B.)** Coefficient of variation was calculated for each test group. Bar graphs represent each individual plate streaked within each group.




B.

Coefficient of Variation

| LP1 LL | LP2 LL | LP1 LN | LP2 LN | Auto |
|---|---|---|---|---|
| 31.10% | 52.07% | 12.17% | 6.20% | 27.94% |

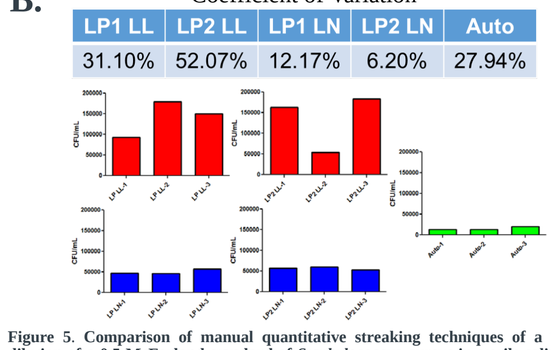
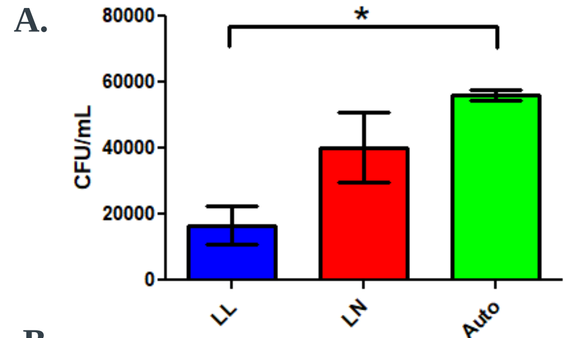


**Figure 5. Comparison of manual quantitative streaking techniques of a 1:1,000 dilution of a 0.5 McFarland standard of *Staphylococcus aureus* in sterile saline. (A.)** Plate group comparison between the "loop-loop" method of streaking (used in Figures 2-4) with the "loop-needle" method. One-way ANOVA $p\leq0.01$**. Asterisks indicate Tukey post-test demonstrating statistical significance between groups $p\leq0.05$* **(B.)** Coefficient of variation was calculated for each test group. Bar graphs represent each individual plate streaked within each group.




B.

Coefficient of Variation

| LL | LN | Auto |
|---|---|---|
| 51.66% | 59.43% | **6.43%** |

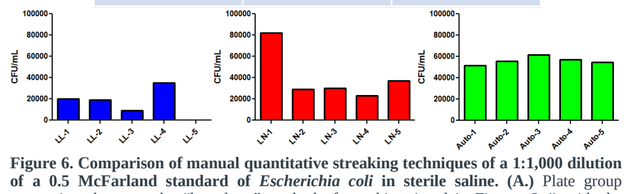


**Figure 6. Comparison of manual quantitative streaking techniques of a 1:1,000 dilution of a 0.5 McFarland standard of *Escherichia coli* in sterile saline. (A.)** Plate group comparison between the "loop-loop" method of streaking (used in Figures 2-4) with the "loop-needle" method. One-way ANOVA $p\leq0.05$*. Asterisks indicate Tukey post-test demonstrating statistical significance between groups $p\leq0.05$* **(B.)** Coefficient of variation was calculated for each test group. Bar graphs represent each individual plate streaked within each group.

## CONCLUSIONS/ DISCUSSION

1. **Manual quantitative streaking of bacterial cultures using a standard 1µl inoculation loop is prone to error.**
   - It has been long established that the delivery of the inoculum volume to the agar plate is inconsistent (1,2).
2. **Inoculation variability exists between different laboratory professionals as well as within the same laboratory professional inoculating multiple, sequential plates.**
   - It has been noted that variability in techniques used (how loop is dipped into inoculum, dip angle, size of sample container, etc.) has an effect on the volume of inoculum delivered to the agar plate upon inoculation (1).
   - Our students noted (not reported or evaluated) that the subjects from the study had different procedures for inoculation (angle of entry, stirring, dipping over an inch into sample, etc.).
   - There is a study showing that there is variability between laboratory professionals when manually inoculating agar plates (3); however, this study does not address the variability between plates inoculated sequentially by the same person.
3. **Automated inoculation of bacterial media demonstrates superior reproducibility to manual methods.**
   - This conclusion reinforces several published studies (3-6).
4. **The loop-needle method of quantitative streaking, preliminarily, appears to be more comparable to automated methods than the loop-loop method.**
   - Further studies need to be performed to assess this method.

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