

**Comparison Study of Utilizing a Reduced Inoculum in
Performing Drug Susceptibility Testing of *Mycobacterium
tuberculosis* to Pyrazinamide using the BACTEC™ MGIT™
960 System at the Missouri State TB Laboratory**

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Focus of presentation

- Importance of reliable PZA susceptibility results and background
- Explain the issues and why the study was conducted
- Approach taken to evaluate the reduced inoculum method
- Testing and Results
- Conclusion
- Where do we go from here

Importance of accurate PZA susceptibility results

- PZA important first-line drug against TB
- Prolongs treatment for TB if PZA-resistant
- Laboratory credibility

Background

- Missouri TB Lab moved into new BSL₃ facility
- MGIT™ replaced BACTEC™ 460
- MGIT™ may over report false PZA-resistance
 - Large inoculum
 - Media pH
 - Poor buffering
- Confirmation of PZA-resistant
 - Repeat testing
 - PZase activity
 - Molecular sequence to assess *pncA* mutation

Background (continued)

- Claudio Piersimoni, et al. proposed

“Laboratories should consider retesting all PZA-resistant isolates to provide accurate and reliable susceptibility results...using reduced inoculum of 0.25-mL”

Purpose of the study

Evaluate PZA susceptibility using a reduced inoculum

Determine whether or not we could incorporate the reduced inoculum into standard practice

Method

- Phase 1: Validate protocol and simultaneously evaluate reproducibility
- Phase 2: Serial dilution study
- Phase 3: Test strains with established PZA results
- Phase 4: Side-by-side comparison

Method (continued)

Phase 1: Validate protocol and simultaneously evaluate reproducibility

➤ Protocol

- ✓ Manufacturers instructions
- ✓ Exception: Test inoculum reduced from 0.5-mL to 0.25-mL
- ✓ Seed tubes prepared and monitored until positive ≥ 4 days; <4 days redo
- ✓ PZA DST performed 1-2 days after positivity
- ✓ Growth control tube inoculated with a 1:10 dilution

Method (continued)

Phase 1: Validate protocol and simultaneously evaluate reproducibility

➤ Reproducibility

✓ *M. bovis* BCG

- Two scientists

- Sets of five over four days

✓ *M. tuberculosis* (ATCC#25177)

- Triplicate

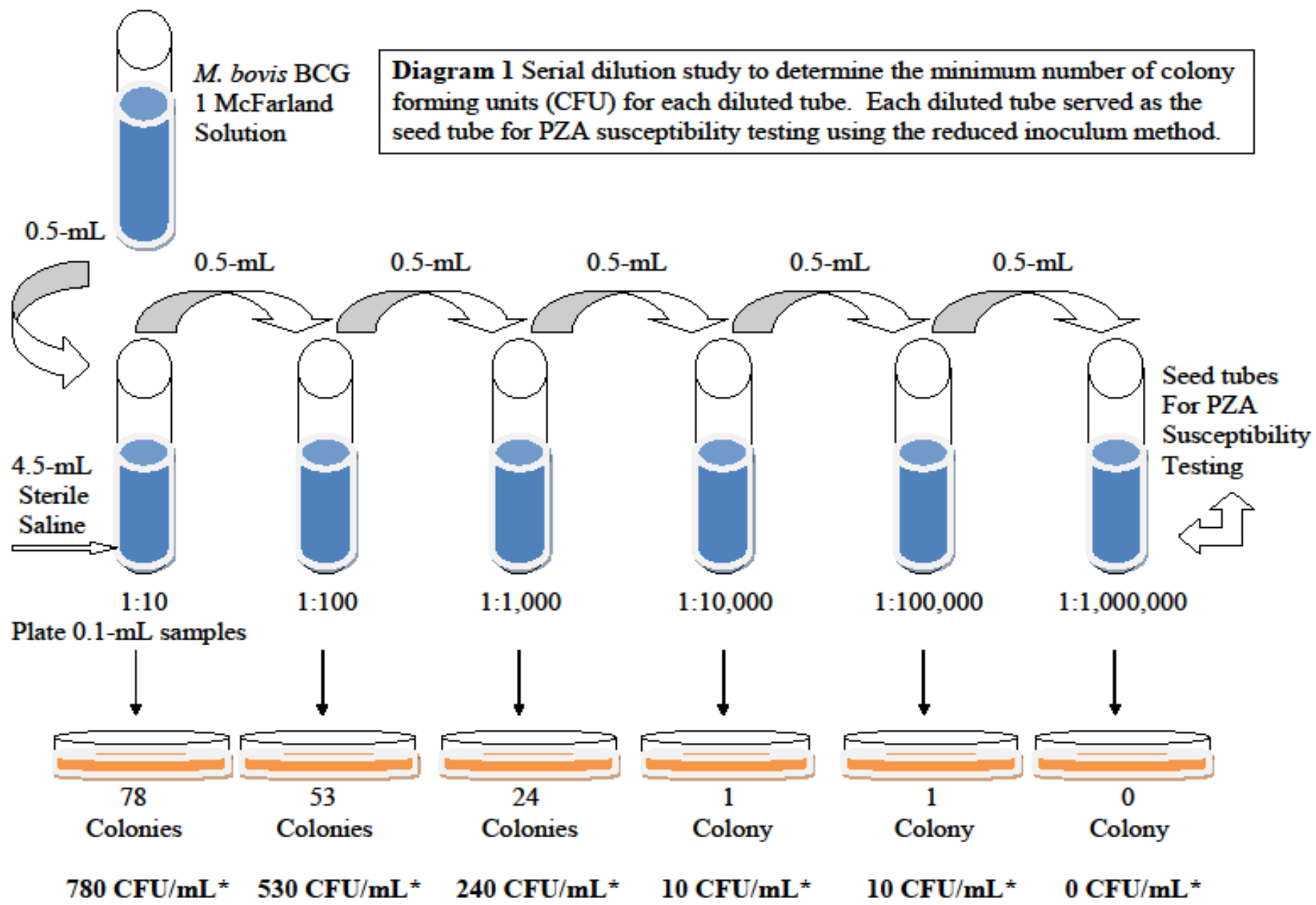
- Performed over two days

Table 1 PZA DST repeatability testing and validation of reduced inoculum protocol using a clinical strain of *M. bovis* BCG and *M. tuberculosis* complex ATCC# 25177

| No. | Specimen Identification | 0.25-mL Inoculum PZA DST Result | Days to DST Result | Expected Result |
|-----|--------------------------------|---------------------------------|--------------------|-----------------|
| A1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| B1 | <i>M. bovis</i> BCG | Resistant | 10 | Resistant |
| C1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| D1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| E1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| A2 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| B2 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| C2 | <i>M. bovis</i> BCG | Resistant | 9 | Resistant |
| D2 | <i>M. bovis</i> BCG | Resistant | 11 | Resistant |
| E2 | <i>M. bovis</i> BCG | Resistant | 9 | Resistant |
| F1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| G1 | <i>M. bovis</i> BCG | Resistant | 6 | Resistant |
| H1 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| I1 | <i>M. bovis</i> BCG | Resistant | 7 | Resistant |
| J1 | <i>M. bovis</i> BCG | Resistant | 6 | Resistant |
| F2 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| G2 | <i>M. bovis</i> BCG | Resistant | 7 | Resistant |
| H2 | <i>M. bovis</i> BCG | Resistant | 7 | Resistant |
| I2 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| J2 | <i>M. bovis</i> BCG | Resistant | 8 | Resistant |
| K1 | <i>M. tuberculosis</i> complex | Susceptible | 7 | Susceptible |
| L1 | <i>M. tuberculosis</i> complex | Susceptible | 10 | Susceptible |
| M1 | <i>M. tuberculosis</i> complex | Susceptible | 7 | Susceptible |
| K2 | <i>M. tuberculosis</i> complex | Susceptible | 7 | Susceptible |
| L2 | <i>M. tuberculosis</i> complex | Susceptible | 7 | Susceptible |
| M2 | <i>M. tuberculosis</i> complex | Susceptible | 7 | Susceptible |

Method (continued)

Phase 2: Serial dilution study



*CFUs were calculated for each respective dilution tube. A decrease by 10 fold was not observed possibly due to the clumping nature of MTBC.

Method (continued)

Phase 2: Serial dilution study (continued)

| Dilution Concentration | DST Result | Days to DST Result | Expected Result |
|------------------------|------------|--------------------|-----------------|
| 1:10 | Resistant | 8 | Resistant |
| 1:100 | Resistant | 13 | Resistant |
| 1:1,000 | Resistant | 18 | Resistant |
| 1:10,000 | Failed* | N/A | Resistant |
| 1:100,000 | Failed* | N/A | Resistant |
| 1:1,000,000 | Failed | N/A | Resistant |

*Growth control tube did not grow within MGIT protocol timeframe; however, growth visible in drug challenge tube and MGIT instrument inventory readings at day 18 were both 400.

Method (continued)

Phase 3: Test strains with established PZA results

Phase 4: Side-by-side comparison

Table 3 MGIT™ PZA DST results compared to the expected results between the standard versus reduced inoculum.

Number of strains with the following results

| PZA DST Inoculum | Total Count | Both-S | Expected-S Inoculum-R | Expected-R Inoculum-S | Both-R | Overall Accuracy (%) |
|------------------------------------|--------------------|---------------|------------------------------|------------------------------|---------------|-----------------------------|
| 0.5-mL (Standard) | 84 | 45 | 10 | 0 | 29 | 88 |
| 0.25-mL (Reduced) | 84 | 52 | 3 | 0 | 29 | 96 |
| Manufacturer (Liquid) ⁶ | 112 | 88 | 1 | 1 | 22 | 98 |

S = Susceptible

R = Resistant

Table 4 MGIT™ PZA DST performance characteristics between the standard versus reduced inoculum to correctly identify PZA resistance

| PZA DST Inoculum | Sensitivity | Specificity | PPV | NPV |
|------------------------------------|--------------------|--------------------|---------------|-------------|
| 0.5-mL (Standard) | 100 (29/29) | 81.82 (45/55) | 74.36 (29/39) | 100 (45/45) |
| 0.25-mL (Reduced) | 100 (29/29) | 94.55 (52/55) | 90.63 (29/32) | 100 (52/52) |
| Manufacturer (Liquid) ⁶ | 96 (22/23) | 98.88 (88/89) | 95.65 (22/23) | 99 (88/89) |

| PZA DST Inoculum | Average Days to Positivity | Range |
|-------------------------|-----------------------------------|--------------|
| 0.5-mL (Standard) | 7.13 | 4 - 13 Days |
| 0.25-mL (Reduced) | 7.93 | 4 - 16 Days |

Table 5 Discrepant results between the two methods using the standard versus reduced inoculum for MGIT™ PZA DST

| Specimen ID | DST Result 0.5-mL Inoculum | Days to result 0.5-mL Inoculum | DST Result 0.25-mL Inoculum | Days to result 0.25-mL Inoculum | Expected Result |
|--------------------|-----------------------------------|---------------------------------------|------------------------------------|--|------------------------|
| G | Resistant | 5 | Susceptible | 5 | Susceptible |
| V | Resistant | 7 | Resistant | 6 | Susceptible |
| W | Resistant | 7 | Susceptible | 8 | Susceptible |
| Y | Resistant | 7 | Susceptible | 8 | Susceptible |
| Z | Resistant | 11 | Resistant | 13 | Susceptible |
| AA | Resistant | 11 | Susceptible | 12 | Susceptible |
| BB | Resistant | 12 | Susceptible | 12 | Susceptible |
| DD | Resistant | 10 | Resistant | 10 | Susceptible |
| HH | Resistant | 9 | Susceptible | 11 | Susceptible |
| II | Resistant | 7 | Susceptible | 9 | Susceptible |

Conclusion

- Reduced inoculum produce comparable results
- Reduced the number of unwarranted repeat testing or forwarding of samples for confirmation by 70%
- Overall accuracy is 96% compared to the standard inoculum (0.5-mL) accuracy of 88%
- No samples tested produced false PZA-susceptible
- PZA-resistance detected at a dilution factor of 1:1000

Future

Low-level Ethambutol (ETH) resistance

- Assuming inoculum size or the number of organisms present
- Study at what point using the reduced inoculum will a PZA-susceptible organism produce a false PZA-resistant result
- Will reducing the inoculum for PZA testing allow a more heavier load of seed tube to identify low-level ETH resistance

References

1. Chedore, P., et al. *Potential for Erroneous Results Indicating Resistance When Using the Bactec MGIT 960 System for Testing Susceptibility of Mycobacterium tuberculosis to Pyrazinamide.* Journal of Clinical Microbiology, 2010. 48(1):300-301.
2. Piersimoni, Claudio, et al. *Prevention of False Resistance Results Obtained in Testing the Susceptibility of Mycobacterium tuberculosis to Pyrazinamide with the Bactec MGIT 960 System Using a Reduced Inoculum.* Journal of Clinical Microbiology, 2013. 51(1):291-294.
3. CLSI. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard – Second Edition.* CLSI document M24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
4. Pfyffer, Gaby E., Frantiska Palicova and Sabine Rüsç-Gerdes. *Testing of Susceptibility of Mycobacterium tuberculosis to Pyrazinamide with the Nonradiometric BACTEC MGIT 960 System.* Journal of Clinical Microbiology, 2002. 40(5):1670-1674.
5. Chang, Kwok Chiu, Wing Wai Yew and Ying Zhang. *Pyrazinamide Susceptibility Testing in Mycobacterium tuberculosis: a Systematic Review with Meta-Analyses.* Antimicrobial Agents and Chemotherapy, 2011. 55(10):4499-4505.
6. BD BACTEC™ MGIT™ 960 PZA Kit [Package Insert]. Becton-Dickinson Co., Sparks, MD; 2009.
7. Centers for Disease Control and Prevention. *Treatment of Tuberculosis,* American Thoracic Society, CDC, and Infectious Diseases Society of America. MMWR 2003; 52(No. RR-11):pg. 37.

Thank you!

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