

# Susceptibility Testing of *Mycobacterium tuberculosis* to Anti-Tuberculosis Drugs: BACTEC™ MGIT™ 960 vs BACTEC™ 460TB System

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## REVISED ABSTRACT

■ Rapid detection of drug-resistant strains of *M. tuberculosis* (*Mtbc*) is essential for efficient management of tuberculosis (TB) patients. For nearly two decades the radiometric BACTEC 460TB System has provided the most rapid method for antimicrobial susceptibility testing (AST). However, the hazards arising from using radioisotopes are obvious. Recently, drug concentrations have been established for AST using the manual version of a broth-based, non-radiometric alternative, the Mycobacteria Growth Indicator Tube (MGIT). We have now evaluated the automated version, the BACTEC MGIT 960, for AST of *Mtbc* to rifampin (RMP) and isoniazid (INH), the two core elements in TB therapy, extended the study to ethambutol (EMB), and streptomycin (SM), and compared the results to the radiometric BACTEC 460TB technology. Prior to testing clinical isolates of *Mtbc* reproducibility of the BACTEC MGIT 960 AST was established by testing a validated panel of 10 *Mtbc* strains in triplicate from 3 separately prepared inocula (thus, 9 replicates per strain). Drug concentrations were 1.0 µg/ml in the BACTEC MGIT 960 and 2.0 µg/ml in the BACTEC 460TB for RMP, 0.1 and 0.4 µg/ml in both systems for INH, 5.0 and 7.5 µg/ml in the BACTEC MGIT 960 and 2.5 and 7.5 µg/ml in the BACTEC 460TB for EMB, and 1.0 and 4.0 µg/ml in the BACTEC MGIT 960 and 2.0 and 6.0 µg/ml in the BACTEC 460TB for SM. In spite of the early stages of evaluation and the possibility that statistical values may be skewed by the limited number of clinical strains tested (n = 38), we obtained excellent agreement for RMP (100%), with 28 strains susceptible (S) and 10 resistant (R) by both systems. The same held true for INH (0.1 µg/ml and 0.4 µg/ml) and EMB (both concentrations). Agreement for SM was, however, lower (86.8%). While the study is still in progress and currently extended to AST of *Mtbc* to pyrazinamide, these preliminary data demonstrate that the BACTEC MGIT 960 System is a promising alternative to the radiometric BACTEC 460TB method and provides the clinician with rapid and reliable AST results.

## INTRODUCTION

Among the novel growth-based strategies for antimicrobial susceptibility testing (AST) is the Mycobacteria Growth Indicator Tube (MGIT). Containing a modified, non-radioactive 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor it allows rapid AST, and thus, overcomes the limitations of radiometry (in particular, safety and regulatory principles). After preliminary studies have been carried out with the manual version of MGIT,<sup>1,3</sup> a fully automated system, the BACTEC MGIT 960, is now available for AST. Positive tubes are flagged by the instrument, i.e., the system's software algorithms evaluate the relative growth in the drug-containing tubes and compare it to the drug-free control tube.

In this study, we have performed AST of *Mtbc* to isoniazid, rifampin, streptomycin, and ethambutol (SIRE) utilizing the BACTEC MGIT 960 System and compared this novel technique to AST done by the radiometric BACTEC 460TB System. Since safety is of prime importance in the clinical mycobacteriology laboratory, we have also evaluated the suitability of plastic tubes.

## MATERIALS AND METHODS

### Study design

#### ■ Phase 1: Reproducibility testing

A panel of 10 characterized strains of *Mtbc* were tested in triplicate from three separately prepared inocula (thus, 9 replicates per strain).

#### ■ Phase 2: Clinical isolate testing

Clinical isolates of *Mtbc* from both solid (Löwenstein-Jensen [LJ]) and liquid sources (MGIT and BACTEC 12B media) were tested in a paired comparison of the BACTEC MGIT 960 System vs the BACTEC 460TB System.

#### ■ Phase 3: Resolution of discrepant results

All isolates (by each source) which yielded discordant susceptibility results with one or the other BACTEC System have been retested by the proportion method on Middlebrook 7H10 agar<sup>4</sup>.

Table 1. Drug Concentrations

| Drug | BACTEC MGIT 960 System (µg/ml) | BACTEC 460 TB System (µg/ml) |
|------|--------------------------------|------------------------------|
| RMP  | 1.0                            | 2.0                          |
| INH  | 0.1/0.4                        | 0.1/0.4                      |
| EMB  | 5.0/7.5                        | 2.5/7.5                      |
| SM   | 1.0/4.0                        | 2.0/6.0                      |

## REFERENCES

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- NCCLS. 1995. Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*; tentative standard.M24-T.

## RESULTS

Table 2. Reproducibility<sup>a</sup>

| Drug (µg/ml)                          | Tests performed (n) | Correct results (n) | % Agreement |
|---------------------------------------|---------------------|---------------------|-------------|
| SM 1.0<br>4.0<br>both concentrations  | 90                  | 89                  | 98.9        |
|                                       | 90                  | 90                  | 100         |
|                                       | 180                 | 179                 | 99.4        |
| INH 0.1<br>0.4<br>both concentrations | 90                  | 90                  | 100         |
|                                       | 90                  | 85                  | 94.4        |
|                                       | 180                 | 175                 | 97.2        |
| RMP 1.0                               | 90                  | 89                  | 98.9        |
| EMB 5.0<br>7.5<br>both concentrations | 90                  | 90                  | 100         |
|                                       | 87                  | 86                  | 98.9        |
|                                       | 177                 | 176                 | 99.4        |
| <b>Grand Total</b>                    | <b>627</b>          | <b>619</b>          | <b>98.7</b> |

\* 10 strains of *Mbc* were tested in triplicate from 3 separately prepared inocula

Table 3. Clinical Isolations — Liquid source<sup>a</sup>

| Drug (µg/ml) |     | n <sup>c</sup>                                      | both - S <sup>d</sup> | 960 - S<br>460 - S | 960 - R<br>460 - R | both - R | Accuracy |
|--------------|-----|---|-----------------------|--------------------|--------------------|----------|----------|
|              |     | Full Agreement of results for SIRE <sup>b</sup> 155 |                       |                    |                    |          |          |
|              |     | Discordant results 6 (3.7%)                         |                       |                    |                    |          |          |
| SM           | 1.0 | 23  | 13                    | —                  | —                  | 10       | 1.000    |
|              | 4.0 | 23  | 15                    | 2                  | —                  | 6        | 0.913    |
| INH          | 0.1 | 23  | 15                    | 1                  | —                  | 7        | 0.957    |
|              | 0.4 | 23  | 16                    | 1                  | —                  | 6        | 0.957    |
| RMP          | 1.0 | 23  | 18                    | 1                  | —                  | 4        | 0.957    |
| EMB          | 5.0 | 23  | 20                    | —                  | —                  | 3        | 1.000    |
|              | 7.5 | 23  | 22                    | 1                  | —                  | —        | 0.957    |

a BACTEC MGIT 960 System: MGIT medium

BACTEC 460TB System: 12B medium

b SIRE, streptomycin, isoniazid, rifampin, ethambutol

c number of strains

d S, susceptible; R, resistant

Table 4. Clinical Isolates — Solid source<sup>a</sup>

| Drug (µg/ml) |     | n <sup>b</sup>                                     | both - S | 960 - R<br>460 - S | 960 - S<br>460 - R | both - R | Accuracy |
|--------------|-----|--|----------|--------------------|--------------------|----------|----------|
|              |     | Full Agreement of results for SIRE <sup>a</sup> 97 |          |                    |                    |          |          |
|              |     | Discordant results 8 (7.6%)                        |          |                    |                    |          |          |
| SM           | 1.0 | 15   | 5        | —                  | —                  | 10       | 1.000    |
|              | 4.0 | 15   | 6        | 5                  | —                  | 4        | 0.667    |
| INH          | 0.1 | 15   | 7        | —                  | —                  | 8        | 1.000    |
|              | 0.4 | 15   | 8        | —                  | —                  | 7        | 1.000    |
| RMP          | 1.0 | 15   | 10       | —                  | —                  | 5        | 1.000    |
| EMB          | 5.0 | 15   | 10       | 1                  | —                  | 4        | 0.933    |
|              | 7.5 | 15   | 13       | 2                  | —                  | —        | 0.867    |

a LJ

b number of strains

Table 5. Clinical Isolates (initial reading) — Combined source<sup>a</sup>

| Drug (µg/ml) |  | <i>n</i> <sup>b,c</sup> | both - S | 960 - R<br>460 - S | 960 - S<br>460 - R | both - R | Accuracy |
|--------------|--|-------------------------|----------|--------------------|--------------------|----------|----------|
| SM 1.0       |  | 38                      | 18       | —                  | —                  | 20       | 1.000    |
| 4.0          |  | 38                      | 21       | 7                  | —                  | 10       | 0.816    |
| INH 0.1      |  | 38                      | 22       | 1                  | —                  | 15       | 0.974    |
| 0.4          |  | 38                      | 24       | 1                  | —                  | 13       | 0.974    |
| RMP 1.0      |  | 38                      | 28       | 1                  | —                  | 9        | 0.974    |
| EMB 5.0      |  | 38                      | 30       | 1                  | —                  | 7        | 0.974    |
| 7.5          |  | 38                      | 35       | 3                  | —                  | —        | 0.921    |

a liquid and solid source

b number of strains

c one third of the strains were tested in MGIT plastic tubes

Table 6. Clinical Isolates (resolved results)<sup>a</sup> — Combined sources<sup>b</sup>

| Drug (µg/ml) |  | <i>n</i> <sup>c,d</sup> | both - S <sup>d</sup> | 960 - R<br>460 - S | 960 - S<br>460 - R | both - R | Accuracy |
|--------------|--|-------------------------|-----------------------|--------------------|--------------------|----------|----------|
| SM 1.0       |  | 38                      | 18                    | —                  | —                  | 20       | 1.000    |
| 4.0          |  | 38                      | 21                    | 5                  | —                  | 12       | 0.868    |
| INH 0.1      |  | 38                      | 22                    | —                  | —                  | 16       | 1.000    |
| 0.4          |  | 38                      | 24                    | —                  | —                  | 14       | 1.000    |
| RMP 1.0      |  | 38                      | 28                    | —                  | —                  | 10       | 1.000    |
| EMB 5.0      |  | 38                      | 30                    | —                  | —                  | 8        | 1.000    |
| 7.5          |  | 38                      | 35                    | —                  | —                  | 3        | 1.000    |

a resolved by the proportion method on Middlebrook 7H10 agar

b liquid and solid source

c number of strains

d one third of the strains were tested in MGIT plastic tubes

Table 7. Clinical Isolates — Time to result (TTR, days)

| Inoculum (day)   | Liquid source   |     |              |     | Solid source    |     |              |     |
|------------------|-----------------|-----|--------------|-----|-----------------|-----|--------------|-----|
|                  | BACTEC MGIT 960 |     | BACTEC 460TB |     | BACTEC MGIT 960 |     | BACTEC 460TB |     |
|                  | <i>n</i>        | TTR | <i>n</i>     | TTR | <i>n</i>        | TTR | <i>n</i>     | TTR |
| 0                | —               | —   | 8            | 6.0 | —               | —   | —            | —   |
| 1 <sup>a</sup>   | 2               | 6.2 | 5            | 5.2 | —               | —   | —            | —   |
| 2 <sup>a</sup>   | 2               | 5.7 | 8            | 5.3 | —               | —   | —            | —   |
| 3 <sup>b</sup>   | 6               | 7.3 | 2            | 5.5 | —               | —   | 1            | 9.0 |
| 4 <sup>b</sup>   | 2               | 7.7 | —            | —   | —               | —   | —            | —   |
| 5 <sup>b</sup>   | 11              | 6.7 | —            | —   | 15              | 9.0 | —            | —   |
| 6                | —               | —   | —            | —   | —               | —   | 14           | 8.8 |
| Total            | 23              | 6.8 | 23           | 5.5 | 15              | 9.0 | 15           | 8.8 |
| Range            | 5.2 - 9.2       |     | 5 - 7        |     | 7.1-11.2        |     | 7 - 12       |     |
| Sources combined | BACTEC MGIT 960 |     | 7.7 days     |     | BACTEC 460TB    |     | 6.8 days     |     |

a MGIT — undiluted

b MGIT — 1:5 diluted

### CONCLUSIONS

- AST by the new, non-radiometric BACTEC MGIT 960 System can be carried out from *Mtbc* strains grown either in liquid or on solid media.
- For both growth and detection as well as for AST MGIT plastic tubes are as suitable as MGIT glass tubes.
- Overall, susceptibility data generated by the new, fully automated BACTEC MGIT 960 System are well comparable to those generated by the radiometric BACTEC 460TB system.
- Concerning time to result there is no statistically significant difference between the two systems (7.7 days [BACTEC MGIT 960] vs 6.8 days [BACTEC 460TB]).
- In being as efficient as the BACTEC 460TB System, the new nonradiometric BACTEC MGIT 960 System is a promising candidate to replace radiometric AST of *Mtbc*.