

# Comparison of the BACTEC™ MGIT™ 320 to the BACTEC MGIT 960 for the Growth, Detection and Susceptibility Testing of *Mycobacterium tuberculosis*.

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## ABSTRACT

**Objective:** Critically compare the BACTEC MGIT 320 to the BACTEC MGIT 960. The MGIT 320 is a new, lower-capacity instrument under development for the growth and detection of *M. tuberculosis* and antimicrobial susceptibility testing using the BACTEC MGIT reagents.

**Methods:** Growth and detection were evaluated in a paired study that included three strains of *M. tuberculosis* and one strain each of *M. avium*, *M. kansasii*, *M. gastri* and *M. marinum*. The study design included both position within the instrument and microbial detection limit as variables. Three dilutions (highest dilution approximately 0 to 10 CFU) per strain were tested. There were 16 replicates per dilution distributed throughout the MGIT 320 or MGIT 960 drawer. Susceptibility testing was compared using one strain in a BD BACTEC MGIT SIRE susceptibility test and two strains in a BD BACTEC MGIT PZA comparison test. A total of 48 AST sets were distributed evenly in both the MGIT 320 and MGIT 960 drawers.

**Results:** A total of 336 paired cultures were analyzed for recovery and time to detection (TTD). There was no difference in total recovery with a McNemar P-value = 1. The recovery rate for the

MGIT 320 and the MGIT 960 was 90.2 and 90.5%, respectively. All recovery failures were with the highest dilution (0 to 10 CFU). The mean TTD in the MGIT 320 and the MGIT 960 was 336 and 341 hours, respectively. The median time to detection difference was 2 hours (N = 290 paired cultures) earlier in the MGIT 320 (not significant by Wilcoxon non-parametric analysis). The percent agreement for all SIRE tests was 99.6%. The difference was a borderline streptomycin determination (43% versus 38% of streptomycin tests determined resistant, 320 and 960 respectively). There was a mean difference in time to result of the SIRE sets of 7 hours (earlier in the MGIT 320). There was 100% agreement and no time in protocol differences for the 32 PZA tests between the two systems. There were no observable differences in growth characteristics based on total Growth Unit (GU) accumulation in the test sets.

**Conclusion:** The data demonstrate that the BD BACTEC MGIT 320 and the BD BACTEC MGIT 960 systems are functionally equivalent for growth, detection and antimicrobial susceptibility testing of *M. tuberculosis*.

## BACKGROUND

Excerpts from recent publications demonstrating the importance of the MGIT 960 as a tool in controlling and hopefully eradicating the global tuberculosis epidemic:

“Global prevalence of MTB infection was 32% (1.86 billion people). Eighty percent of all incident TB cases were found in 22 countries, with more than half the cases occurring in 5 Southeast Asian countries. Nine of 10 countries with the highest incidence rates per capita were in Africa. Prevalence of MTB/HIV coinfection worldwide was 0.18% and 640,000 incident TB cases (8%) had HIV infection. The global burden of tuberculosis remains enormous, mainly because of poor control in Southeast Asia, sub-Saharan Africa, and eastern Europe, and because of high rates of *M. tuberculosis* and HIV coinfection in some African countries.”

Dye, C., S. Scheele, P. Dolin, V. Pathania, and M. C. Raviglione. 1999. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence and mortality by country. WHO

Global Surveillance and Monitoring Project. JAMA. 282:677-868.

“Of 2566 specimens received from October 2004 to September 2006, 1355 (53%) were culture positive by MGIT compared with 1013 (39%) by LJ. Median time to growth for MGIT was significantly less than LJ: 11 versus 27 days. Of 1417 isolates detected by at least 1 media, 1255 (86%) were identified as MTB and 162 (11%) NTM. MGIT improved speed and sensitivity of MTB isolation and drug susceptibility testing, regardless of HIV status.”

Srisuwanvilai, L, Monkongdee, P., Podewils, L. J., Ngamlert, K., Pobkeeree, V., Puripokai, P., Kanjanamongkolsiri, P., Subhachaturas, W., Akarasewi, P., Wells, C. D., Tappero, J. W. and Varma, J. K. (2008) Performance of the BACTEC MGIT 960 compared with solid media for detection of *Mycobacterium* in Bangkok, Thailand. *Diag Microbiol & Infect Disease*. 61: 402-407.

## BACKGROUND (CONTINUED)

These quotations from recent publications demonstrate the fundamental importance of liquid culture and phenotypic drug susceptibility testing (DST) as part of a complete strategy in the ongoing global efforts to combat tuberculosis. Culture and DST confirmation testing offers an affordable approach to diagnosis of disease and is critical to monitoring epidemiology and the development of drug resistance within infected populations. The BACTEC MGIT 960 TB diagnostic culture system provides a valuable tool for growth, detection and DST testing of *Mycobacterium tuberculosis* from primary culture isolation. The BACTEC MGIT 320 is a new instrument developed as a low culture throughput alternative to the MGIT 960 instrument.

This instrument is specifically designed to provide the same test menu, performance and ease of use as the MGIT 960 system in a smaller format. The MGIT 320 uses the same reagents and evaluates and reports growth in the same manner as the MGIT 960 system. It is the objective of this study to demonstrate that the instrument incubates, interrogates and reports equivalent results on cultures (Growth and DST) in the system. The study is designed to demonstrate reproducibility of the interpretation of growth in the reagents both within the drawer (spatial location) and between instruments (comparing the MGIT 320 to the MGIT 960).

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## STUDY DESIGN

The study is a comparison of biological performance and reproducibility of the BACTEC MGIT 320 instrument to the BACTEC MGIT 960. The study design uses multiple paired test sets (split between instruments) that are distributed throughout the test drawer of each instrument to demonstrate biological performance reproducibility with a 95% confidence level. Three *M. tuberculosis* strains and four non-tuberculosis mycobacteria (*M. avium*, *M. gastri*, *M. kansasii* and *M. marinum*) were inoculated into MGIT tubes at three dilution levels (0 to 10, 10 to 100 and 100 to 1000 CFU/tube) in replicates of 16 at each level. The MGIT tubes were supplemented with MGIT Growth Supplement and MGIT PANTA™ per the manufacturer's instructions for primary isolation from sputa. The inoculated tubes were then distributed throughout the drawer of the test instrument (MGIT 320) such that each strain and inoculum is represented in all areas within the drawer (front back center, left, right etc.). The paired tube for analysis is the same strain, inoculum and position in the control (MGIT 960) drawer. A similar array of MGIT SIRE (one strain) and MGIT PZA (two

strains) DST sets was created in the two drawers. Analysis of the data is based on system output (growth unit, positive, negative, susceptible or resistant) using the following statistical methods when appropriate, McNemar chi Square for recovery and a Wilcoxon paired analysis for time to detection.

Organisms: *Mycobacterium tuberculosis* strains ATCC™ 25177 (H37RA), CDC E23 and CDC E30 (strains obtained from the CDC in 1999) were used in the growth and detection portion of the study. *M. tuberculosis* strains BD 201, CDC E3 and ATCC 35828 were used in the drug susceptibility portion. Inoculum suspensions were made by standard procedures and controlled by plate count using Middlebrook 7H10 Agar plates.

The following MGIT reagents were used in this study: MGIT Mycobacteria Growth Indicator Tubes (7 mL), MGIT Growth Supplement, MGIT PANTA, MGIT 960 SIRE Susceptibility Test Kit and the MGIT PZA Susceptibility Test.

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## RESULTS

There was no difference in total recovery between the MGIT 320 and the MGIT 960 ( $P = 0.823$ ). The recovery rate of the 336 paired sets for the MGIT 320 and the MGIT 960 was 94.9% and 94.3%, respectively (a difference of two cultures). The total number of paired positive sets was 308 with a Wilcoxon median difference 2 hours earlier in the MGIT 320 (confidence interval was -7.5 to 3 hours). Three *M. avium*, three *M. kansasii* and one *M. gastri* failed to detect in the MGIT 320. Five *M. avium*, three *M. kansasii* and two *M. gastri* failed to detect in the MGIT 960. All recovery failures were at the highest dilution (0 to 10 CFU/tube). These failures can be attributed to low inoculum. The mean TTD for all positive cultures in the MGIT 320 and MGIT 960 system is 14.4 days and 14.5 days, respectively.

The mean TTD of all paired *M. tuberculosis* cultures that detected in the MGIT 320 and MGIT 960 is 14.8 days and 15.1 days, respectively. The mean TTD of the *M. tuberculosis*

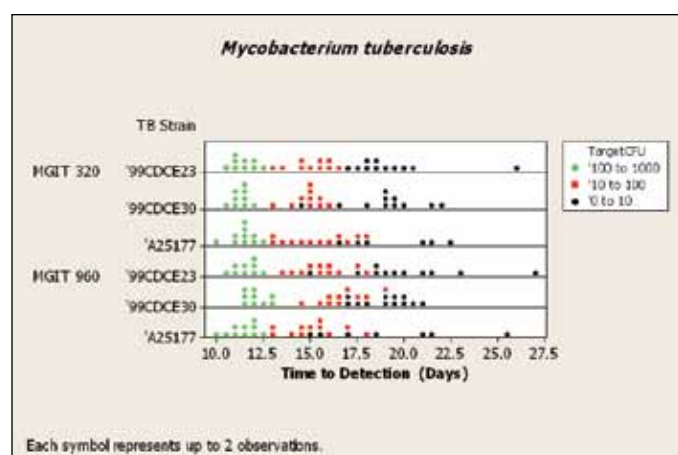
cultures inoculated with 0 to 10 CFU is 19.0 days and 19.1 days in the MGIT 320 and MGIT 960, respectively. The mean TTD difference attributable per log inoculum for *M. tuberculosis* is 3.75 days with both systems being 0.05 days (1.2 hours) from the mean. The mean TTD for the non-tuberculosis strains in the MGIT 320 is 17.8 days (*M. avium*), 20.0 days (*M. gastri*), 11.3 days (*M. kansasii*) and 14.8 (*M. marinum*). The mean difference in TTD comparing the MGIT 320 to the MGIT 960 for these mycobacteria ranged from 3 to 14 hours and they were not significantly different by Wilcoxon analysis with 95% confidence. The TTD data for all positive paired cultures is summarized by strain in Table 1. The TTD data for *M. tuberculosis* that shows the effect of inoculum dilution is presented in Figures 1 and 2 and for the non-tuberculosis bacteria in Figures 3 and 4. The data presented here differs from the original abstract due to late protocol detection of *M. gastri* at the 0 to 10 CFU dilution.

RESULTS (CONTINUED)

**Table 1.** Summary of Time to Detection for all positive paired cultures. Data includes all three inoculum dilutions.

Strain	N	Mean TTD in Days (StDev)		
		MGIT 320	MGIT 960	Difference
<i>M. tuberculosis</i> CDC23	48	15.1 (3.4)	15.6 (3.7)	0.5
<i>M. tuberculosis</i> CDC30	48	15.1 (3.3)	15.9 (3.0)	0.8
<i>M. tuberculosis</i> H37RA	35	13.9 (3.1)	13.4 (2.3)	0.5
<i>M. avium</i> 2675	41	17.8 (6.7)	18.0 (7.0)	0.2
<i>M. gastri</i> 2972	45	20.2 (4.5)	19.5 (5.3)	0.7
<i>M. kansasii</i> 12478	43	11.3 (2.1)	11.5 (2.4)	0.2
<i>M. marinum</i> 2336	48	7.9 (2.8)	7.6 (2.6)	0.3

**Figure 1.** The individual time to detection of the growth and detection cultures of *Mycobacterium tuberculosis* in the two BACTEC MGIT systems.



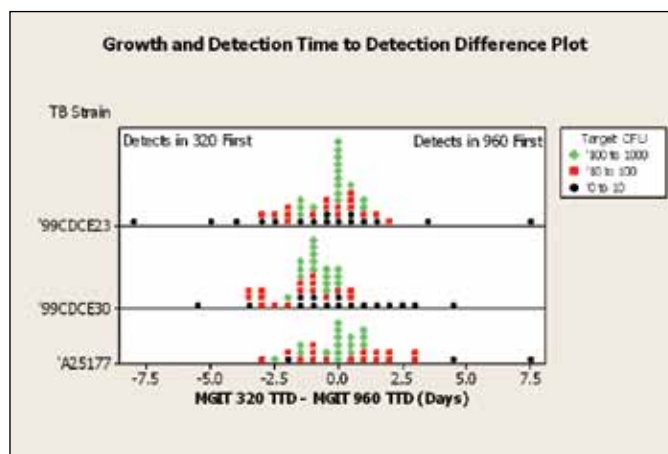
No significant differences were observed in the time to result (TTR) in the drug susceptibility test. A total of 48 test sets were performed. Three strains were tested (1 with SIRE and 2 with PZA) each with a total of 16 paired sets. The sets were randomly distributed throughout the drawers and analyzed as pairs based on location.

The SIRE test strain (CDC E3) is a slow-growing multidrug-resistant strain that is known to demonstrate a borderline result at the low streptomycin test concentration. The mean TTR was 7 hours earlier in the MGIT 320 instrument (7.9 versus 8.2 days in the MGIT 960). The Wilcoxon median difference in TTR was 0.25 days (6 hours) earlier in the MGIT 320 (not significant). There was 100% agreement between the systems on the IRE results (all resistant). There was equivalence on the streptomycin result (7 of 16 and 6 of 16 for the MGIT 320 and 960, respectively). Analysis of the GU accumulation from day 7 to 9 indicates that any difference between the two systems at estimating growth during this critical time period for determining drug susceptibilities is minor.

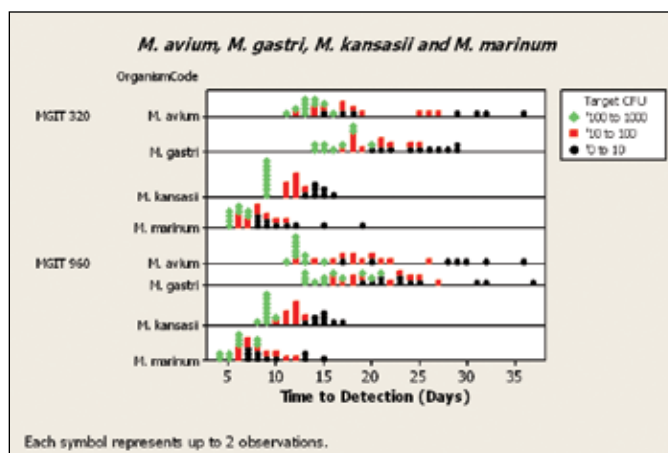
Two strains were tested with PZA, one sensitive (BD 201) and one resistant (ATCC 35828). There was no difference in TTR between the two systems with the average over time in protocol of 5.6 days. There was 100% agreement in the PZA test results between the two instruments.

DST results are summarized in Table 2 and presented in Figure 5.

**Figure 2.** The paired time to detection difference plot of the growth and detection of *M. tuberculosis* cultures in the two BACTEC MGIT systems. The distribution of the difference in time to detection is centered at or near zero indicating little or no difference between the systems.

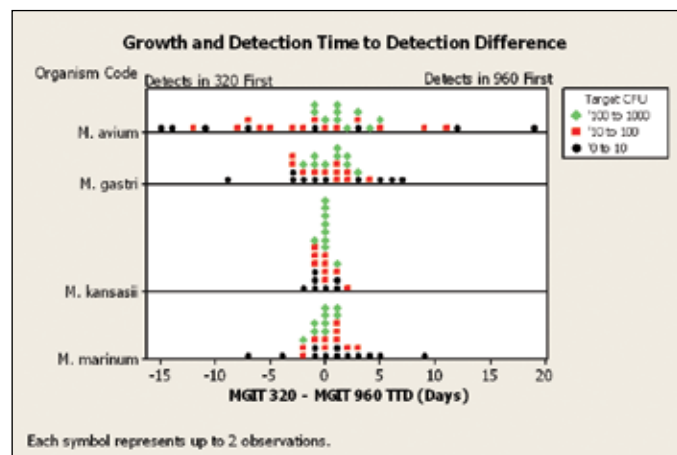


**Figure 3.** The individual time to detection of the growth and detection cultures of non-tuberculosis mycobacteria cultures in the two BACTEC MGIT systems.

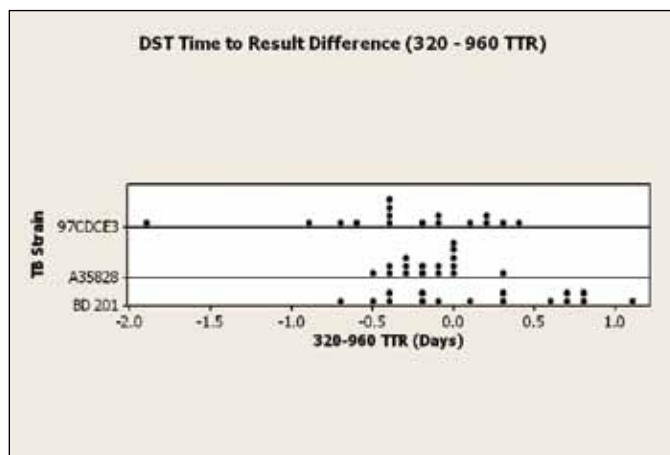


## RESULTS (CONTINUED)

**Figure 4.** The paired time to detection difference plot of the growth and detection of non-tuberculosis mycobacteria cultures in the two BACTEC MGIT systems. The distribution of the difference in time to detection is centered at or near zero indicating little or no difference between the systems.



**Figure 5.** Time to result for the drug susceptibility test comparison of the two BACTEC MGIT systems. The distribution of the difference in time to result is centered at or near zero indicating little or no difference between the systems.



**Table 2.** Time to result analysis of the DST comparison between the MGIT 320 and the MGIT 960 systems.

Test System	Strain	DST	N	Time to Result (Days)				
				Mean	StdDev	Median	Min	Max
MGIT 320	CDCE3	SIRE	16	7.9	0.3	8.0	7.0	8.4
MGIT 960	CDCE3	SIRE	16	8.2	0.3	8.2	7.7	8.9
MGIT 320	BD 201	PZA	16	5.7	0.4	5.1	5.9	6.0
MGIT 960	BD 201	PZA	16	5.5	0.2	5.5	5.7	6.5
MGIT 320	ATCC 35828	PZA	16	5.5	0.2	5.5	5.2	5.7
MGIT 960	ATCC 35828	PZA	16	5.6	0.2	5.6	5.2	5.9

## DISCUSSION

Biological systems and particularly growth and detection based systems will have performance variability. The ideal system will minimize and control the inherent variability in the system for optimal performance. The purpose of this study was to analyze the overall variability of the new MGIT 320 system in comparison to the current MGIT 960 system. The study was designed to test the fundamental purpose of the system which is the analysis of oxygen consumption using a fluorescent sensor as an indicator and convert this information into a quantitative growth metric (the Growth Unit

or GU) which is used to determine vial status (positive or negative) or to compare the quality of growth within culture sets to determine the level of antibiotic sensitivity of an *M. tuberculosis* isolate (drug susceptibility testing). The data presented in this study using multiple replicates of cultures and controlled reagents throughout the test system (MGIT 320) in a direct paired analysis to the control system (MGIT 960) indicate that the MGIT 320 is equivalent for the performance of all tests and assays currently performed in the MGIT 960.

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ATCC is a registered trademark of the American Type Culture Collection.

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