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Comparison of BACTEC MGIT 960 system and BACTEC 460 TB system for growth and detection of *Mycobacteria* from clinical specimens

R GANESWRIE *MBBS, MPath,* CS CHUI *MIMLS,* S BALAN *MBBS, MMedAnes, and* *SD PUTHUCHEARY *MBBS, FRCPath*

*Hospital Sultanah Aminah, Johor Bahru and *Department of Medical Microbiology, Faculty of Medicine, University of Malaya.*

Abstract

This study was carried out to compare the performance of BACTEC MGIT 960 with the BACTEC 460 TB for growth and detection of *Mycobacteria* from human clinical specimens. The BACTEC MGIT 960 instrument is a fully automated system that utilizes MGIT tubes containing an oxygen sensor embedded in silicon at the bottom and filled with 7 mL of modified Middlebrook 7H9 broth. Identical samples were inoculated into the two automated systems and incubated for six weeks. Over a period of three months, 279 specimens were decontaminated and processed according to the standard CDC NALC/NaOH method, using the commercial MycoPrep kit. Forty-two specimens (15%) yielded *Mycobacterium tuberculosis*; 37 (88%) were detected by the fluorescent BACTEC MGIT 960 and 35 (83%) detected by the radiometric BACTEC 460 TB. Fifteen specimens (5%) yielded *Mycobacterium* Other Than Tuberculosis (MOTT); 10 (66%) were detected by BACTEC MGIT 960 and 11 (73%) detected by BACTEC 460 TB. The average time to detection and contamination rates and the average time to obtain results of antimicrobial susceptibility tests between the two systems were compared. The performance of the BACTEC MGIT 960 was comparable to the BACTEC 460 TB system which has been the "Gold Standard" for automated detection of TB. The former was more rapid, as sensitive and less labour intensive than the BACTEC 460. Our data demonstrates that the BACTEC MGIT 960 system is an accurate, automated and a non-radioactive alternative to the BACTEC 460 TB for the culture and susceptibility testing of *M. tuberculosis*.

Key words: Tuberculosis, BACTEC, rapid detection.

INTRODUCTION

Tuberculosis (TB) is a significant worldwide problem and one of the leading causes of morbidity and mortality caused by an infectious agent.[1] In 1993, the World Health Organization (WHO) acknowledged the magnitude of the problem by declaring TB to be a "Global Emergency", the first disease to be so classified in the history of WHO.[2] The rising incidence of tuberculosis and other mycobacterial diseases has made it essential for technological advances in the clinical microbiology laboratory that can detect and identify *Mycobacteria* rapidly. Since its introduction, the BACTEC 460 TB system (Becton Dickinson, Sparks, Maryland) has been widely accepted as the "gold standard" as it can dramatically reduce the detection time to less than two weeks.[3,4] as compared to the traditional culture methods which may require up to six weeks for detection alone. However, this system has several drawbacks, such as the use of radioactive materials, a labour-intensive workflow, lacking an on-board incubation facility and the potential risk of cross-contamination.[5] Furthermore, the use of needles for inoculation of the vials involves the risk of needle stick injury. Recently, the BACTEC MGIT 960 system (Becton Dickinson) has been developed to overcome the pitfalls of the BACTEC 460 with the primary aim of detecting *Mycobacteria* and to carry out susceptibility testing. It is a non-radiometric, fluorescence based, continuous monitoring detection system that measures bacterial growth by determining the oxygen consumption during microbial metabolism.

We carried out a prospective study over a period of three months to compare the performance of the BACTEC MGIT 960 and the

Address for correspondence and reprint requests: SD Puthucheary, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

BACTEC 460 TB system with regards to growth, detection and susceptibility testing of *Mycobacterium tuberculosis* complex.

MATERIALS AND METHODS

The investigation was carried out on 279 human clinical specimens (204 respiratory and 75 non-respiratory) received with a request to determine the possible presence of *Mycobacteria.* The specimens were digested and decontaminated by means of the standard CDC NALC/NaOH method using the commercial MycoPrep kit (Becton Dickinson). Smears were prepared from the final suspension and stained using the Ziehl-Neelson (Z-N) technique. Aliquots of 0.5ml of the decontaminated specimen were inoculated into the BACTEC 12B vials followed by the MGIT 960 tubes. Prior to inoculation, the BACTEC 12B vials and MGIT tubes were supplemented with a mixture of polymixin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin (PANTA), along with growth supplement. The MGIT 960 tubes were incubated in the BACTEC MGIT 960 instrument while the BACTEC 460 12B vials were incubated in a 37°C incubator and manually loaded at weekly intervals onto the BACTEC 460 instrument to be read**.** The incubation was carried out for a maximum of six weeks.

MGIT 960 tubes that had a positive signal with the instrument and BACTEC 12B vials with a Growth Index of ≥ 100 were confirmed as positive after carrying out a smear and acid-fast stain. Positive signals that failed to reveal acid-fast bacilli in smears were screened by Gram staining and if positive**,** eliminated as a contaminant. *Mycobacteria* detected in the BACTEC MGIT 960 system were identified using nucleic acid probes (BD ProbeTec ET). This nucleic acid amplification system utilizes Strand Displacement Amplification (SDA) technology, allowing rapid and real time fluorescent detection of the target organism DNA (*IS6110*). The system provides an internal amplification control to monitor potential inhibitors and results can be achieved within three hours. In addition, this method allows rapid identification of *Mycobacteria* directly from the processed sputum samples. The *Mycobacteria* detected in BACTEC 460 TB system was identified using the BACTEC NAP (p-nitro-α-acetyl-β-hydroxypropiophenone) differentiation test (Becton Dickinson). Antimicrobial suscebtibility testing was carried out on cultures obtained from both the systems using the four first tier antituberculous drugs, which are streptomycin, isoniazid, rifampin and ethambutol.

RESULTS AND DISCUSSION

Forty-seven isolates of *Mycobacteria* were recovered from 279 specimens from respiratory and non-respiratory samples during the three month study period. There were 42 strains of *M. tuberculosis* complex and 15 *Mycobacteria* other than tuberculosis (MOTT) which were all *M. avium* complex. The BACTEC MGIT 960 recovered 37 isolates (88%) of *M. tuberculsosis* and 10 isolates (66%) of MOTT. The BACTEC 460 TB, on the other hand, recovered 35 isolates (83%) of *M. tuberculosis* and 11 isolates (73%) of MOTT. Six isolates of *M. tuberculosis* were recovered only in the BACTEC MGIT 960 and four only in the BACTEC 460 TB. Similarly, four isolates of MOTT were recovered only from the BACTEC MGIT 960 and five only from the BACTEC 460 TB. The possible reasons for the recovery of isolates from the MGIT 960 tubes and not the BACTEC 460 12B vials may be that the *Mycobacteria* that was present did not metabolize the ^{14}C palmitic acid in BACTEC 12B vials, or that the higher volume of media in the MGIT 960 tubes diluted out the potential growth inhibitors, thus providing a better opportunity for the recovery of *Mycobacteria*.[6] On the other hand, there were some cultures which were only positive in the BECTEC 12B vials, and the possible reasons for this may be the aliquots were inoculated first into the BACTEC 12B vials followed by the MGIT 960 tubes. The possible uneven distribution of organisms in the specimens and the random inoculation of the two systems could have accounted for the differences in the yield of *Mycobacteria*. However, our study, in accordance with findings of other authors[7] revealed that neither system recovered all the isolates of *M. tuberculosis* complex or MOTT. A major setback in our study was not to combine the liquid medium with the traditional solid media as recommended by CDC for the best recovery of *M. tuberculosis* and MOTT.[8,9,10]

The average time to detection for the different species of *Mycobacteria* isolated was less with the BACTEC MGIT 960 compared to the BACTEC 460 TB. The three month average time to detection for *M. tuberculosis* was 8.5 days with the BACTEC MGIT 960 and 11.4 days with the BACTEC 460 TB system (Table 1). On the other hand, the three month average time for MOTT was 8.4 days with the BACTEC

TABLE 1: Average days to detection of *Mycobacteria* by individual system

Organism group	Average days to detection							
	BACTEC MGIT 960				BACTEC 460 TB			
	Aug	Sep	Oct	3 month average	Aug	Sep	Oct	3 month average
M. tuberculosis complex	7.3	9.5	8.2	8.5	9.2	11.6	13.2	11.4
MOTT	-	10.7	4.5	8.4	-	12	5	9.9

MGIT 960 and 9.9 days with the BACTEC 460 TB. The time to detection of *Mycobacteria* is a vital performance characteristic of detection systems, as rapid diagnosis of tuberculosis is highly relevant to limit the transmission and contain the spread of tuberculosis.[11]

Overall, mycobacterial growth was detected from smear positive and smear negative specimens in each of the system. Table 2 summarizes the average days to detection and range of times for mycobacterial growth based on the initial smear results. The mean recovery time of *M. tuberculosis* from smear negative specimens was significantly faster (8.5 days) with the BACTEC 960 than the BACTEC 460 (12 days) and both systems were equally sensitive in detecting *M. tuberculosis* from smear negative samples as reported by Saleh *et al*.[12]

During the course of this study, it was noticed that the BACTEC MGIT 960 medium showed higher contamination rate compared to the BACTEC12B medium (Table 3) which may be attributed to the more enriched medium in the MGIT 960 system. The medium used in the BACTEC 460 TB system relies on the metabolic utilization of radiolabeled palmitic acid for detection of ^{14}C-labeled CO_2, signaling the presence of growing *Mycobacteria;* this medium is very low in nutrient content as the radiolabeled substrate is not an optimal growth environment for bacteria that do not use the ^{14}C substrate, a probable reason for a lower contamination rate. As with any new technology, the contamination rate for MGIT 960 medium was exceptionally high during the first month (12.6%) of study but fell significantly (5.1%) in the subsequent months. Although no changes were made to the digestion/decontamination procedure, the recommended contamination rate of 2 to 5% was achieved.[13] This may be attributed to the improved handling techniques by the laboratory personnel involved. Recovery of mixed mycobacterial isolates from the positive MGIT tubes were re-processed (digestion/decontamination) and inoculated into fresh MGIT tubes.

Cultures of *Mycobacteria* were smeared and stained from by the Z-N stain**.** Subsequent identification of *M. tuberculosis* and MOTT was carried out using a molecular method, BD ProbeTec ET system (Becton Dickinson) which yielded results within four hours. On the other hand, positive cultures from BACTEC 12B vials were differentiated using the NAP test, which usually takes up to four days for confirmation. Hence, there was a significant reduction in identification time of mycobacterial species using the MGIT 960 compared to the BACTEC 460.[14] Besides detecting and identifying mycobacterial species, susceptibility testing of four primary

TABLE 2: Time to detection and recovery based on smears for acid fast bacilli.

Smear results		BACTEC MGIT 960		BACTEC 460 TB	
	n	mean	range	mean	range
positive	40	6	4-8	8.3	5-11
negative	3	8.5	3-19	12	5-37

n= number

TABLE 3: Contamination rates (%) for the individual system

Culture System	Aug	Sep	Oct	3 month average
BACTEC MGIT 960	12.6	5.8	5.1	7.8
BACTEC 460 TB	5.8	4.1	7.8	5.9

drugs (streptomycin, isoniazid, rifampin and ethambutol) against *M. tuberculosis* complex was also compared for the two culture systems. A total of 31 tests were performed and the results from BACTEC MGIT 960 system were in accordance with the BACTEC 460 TB system. The average time to result was 8.4 days with BACTEC MGIT 960 and 9.9 days with BACTEC 460 TB, while the total time to antimicrobial susceptibility results ranged from 4 - 10 days and 5-12 days respectively.

These data indicate that the performance of BACTEC MGIT 960 system was comparable to the BACTEC 460 TB system. The average time to detection for positive cultures and susceptibility testing in BACTEC MGIT 960 was significantly less compared to the latter system and might save up to one week to complete the identification and the sensitivity testing. Thus, in line with the recommendations by the Centers for Disease Control and Prevention, using the BACTEC 960 for detection, identification and sensitivity testing of *M. tuberculosis* complex can be made available within 14, 21 and 30 days from specimen collection.[15]

CONCLUSION

The results reported here substantiate the fact that the BACTEC MGIT 960 system is equivalent to the radiometric BACTEC 460 TB system, as the recovery rates were comparable, while time to detection for all *Mycobacteria* was significantly reduced using the BACTEC MGIT 960. However, the fluorescent-based system eliminates the use of hazardous radioactive materials and minimizes the risk of bottle breakage with the use of plastic MGIT tubes. Furthermore, the BACTEC MGIT 960 is a fully automated, non-invasive system that reduces the risk of cross contamination and carry over and eliminates the use of needles. The identification of samples by means of a bar code eliminates the risk of transcription errors while the maintenance of the instrument is minimal.

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