

A comparative study for the detection of *M tuberculosis* by BACTIC MGIT 960 and Lowenstein Jensen media

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Abstract

Tuberculosis remains a major public health problem world-wide. There is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens to hasten the administration of appropriate antimycobacterial therapy and prevent the spread of infection in the community.

The present study aimed to evaluate the performance of BACTEC MGIT 960 as a fully automated, rapid method. A total of 150 sputum specimens were collected from patients suspected to have pulmonary tuberculosis who attended National Tuberculosis Control Program and the Reference TB Laboratory in Sana'a city. All specimens were digested and decontaminated by the N-acetyl-L-cysteine-NaOH method. After decontamination, they were inoculated into BACTEC MGIT 960 and Lowenstein Jensen media. All the inoculated media were incubated at 37°C for the suitable periods.

*Out of 150 specimens, 21(14.0%) *M tuberculosis* were detected with both BACTEC MGIT 960 and Lowenstein Jensen. As a single method, 21(14.0%) were detected by BACTEC MGIT 960 and 20 (13.3) with Lowenstein Jensen medium. The recovery rates were 100 (21/21) with BACTEC MGIT 960 and 95.2% (20/21) with Lowenstein Jensen. Time to detection (TTD) for *M. tuberculosis* were 10.1 days with BACTEC MGIT 960 and 23.7 days with Lowenstein Jensen medium. The difference in TTD was statistically significant ($x = 41$, $P > 0.001$). In conclusion, the BACTEC MGIT 960 system is a rapid and more suitable method for detection of *M tuberculosis*. It also increases the overall recovery of *M tuberculosis* in culture.*

Introduction:

Tuberculosis (TB) remains a major public health problem world-wide. About 8 to 10 million new cases and 2 to 3 million deaths are notified each year.(Kalafati - Tzimaka et al, 2008)(1).The spread of HIV/AIDS and emergence of multiple drug-resistant TB have further contributed to the worsening impact of the disease (2). The incidence of tuberculosis is high in Yemen and remains a serious public health problem. Moreover, the appearance of multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB) has increased the need for rapid diagnostic methods(3).

Although direct AFB microscopy and conventional Lowenstein Jensen (LJ) culture remain the cornerstone for the diagnosis of TB, the sensitivity of these traditional methods is quite low, especially in the samples containing small numbers of organisms (4).

There is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens to hasten the administration of appropriate antimycobacterial therapy and prevent the spread of infection in the community. A variety of manual and automated systems have been developed specifically to reduce the time to detect and identify *Mycobacteria* (5).

The best liquid culture system, BACTEC 460 can detect mycobacterial growth within 14 days (6,7). However, due to the need to dispose of the radioactive materials, this system does not have broad application in Yemen. The manual *Mycobacteria* Growth Indicator Tube (BBL MGIT) culture system was developed to overcome this problem, and many studies have shown its efficiency (8,9,10).

Later, the BACTEC MGIT 960 system, a fully automated, rapid, high-capacity, non-radiometric system, became

available. This system contains a modified Middle brook 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor (silicon rubber impregnated with ruthenium pentahydrate) in an atmosphere of 10% CO₂. The BACTEC MGIT 960 system automatically records bacterial growth every 60 minutes based on O₂-sensitive fluorescence (11,12,13). Only few reports have been published to evaluate this new system. The present study was aimed to evaluate the performance of BACTEC MGIT 960 and to compare the recovery rate and the mean time to detection (TTD) with Lowenstein Jensen medium.

Materials and Methods

A total of 150 sputum specimens were collected from patients suspected to have pulmonary tuberculosis who attended National Tuberculosis Control Program and the Reference TB Laboratory in Sana'a city. All specimens were digested and decontaminated by the N-acetyl-L-cysteine–NaOH method as described by Kent and Kubica (14). After decontamination, smears were prepared from the concentrated sediments of the specimens for Ziehl-Neelsen stain (ZN) (acid-fast staining). The remaining sediment of each specimen was suspended in 1.5 ml of sterile phosphate-buffered saline (pH 6.8). Before inoculation, BACTEC MGIT 960 was supplemented as described by the manufacturer. 0.5 ml of the processed specimen was inoculated into BACTEC MGIT 960 and 0.2 ml onto each of two LJ medium slants. All inoculated media were incubated at 37°C. BACTEC MGIT 960 vials were introduced into the BACTEC MGIT 960 instrument as recommended by the manufacturer and tested either until they were found to be positive or for 6 weeks. The Time to detection (TTD) of mycobacterial growth was determined on the date of the earliest positively which correlated with AFB smear positively. The L J medium slants were examined twice weekly for the first week and weekly for 7 weeks for the visible appearance of colonies and the TTD was based on the earliest date of detection of colonies. After confirmation of mycobacterial growth in a liquid or solid medium. On the day of detection, all

positive liquid and solid media were examined by ZN staining to confirm the presence of acid-fast bacteria (AFB) and subcultured onto blood agar to check for contaminants. Cultures found AFB positive by microscopy were identified by means of conventional biochemical tests (15).

Results

The results of this study that was performed to evaluate a new automated BACTEC MGIT 960 system are illustrated in detail in the following

Table (1) Number and isolation rates of *M. tuberculosis* complex by BACTEC MGIT 960 system and Lowenstein Jensen medium and Contamination rate

<i>M tuberculosis</i> isolated										
Media	Smear positive		Specimens (158)				Contamination rate			
			(No 8)		Smear negative		(No 150)		Total	
	No	%	No	%	No	%	No	%		
-BACTEC MGIT 960	8	100	13	9.15	21	14.00	6	4.00		
-L owentein Jensen	8	100	12	8.45	20	13.33	4	2.66		
-Both	8	100	13	9.15	21	14.00	10	6.66		

There was no statistically significant difference in isolation between both media ($x^2 = 0.1$, $p = 0.74$).

Table 1 shows that of the 150 specimens tested, 21 (14.00 %) were culture positive for *M tuberculosis* in both media, of which 8 (100 %) were smear-positive and 13 (9.15 %) were smear-negative. The contamination rates with BACTEC MGIT 960 was 6/150 (4.00 %) and with LJ found to be 4/150 (2.66 %). There was no statistically significant difference in isolation between BACTEC MGIT960 instrument and L J medium ($x^2 = 2.15$, $P = 0.74$).

(Table 2) Recovery rates of *M tuberculosis* by BACTEC MGIT 960 system compared with Lowenstein Jensen medium

Recovery rate of <i>M tuberculosis</i> complex				
Media	Smear positive		Smear negative	
	(No 8)	No	(No 13)	No
	No	%	No	%
-BACTEC MGIT 960	8	(100/8)8	(13/13)100	13
-L owentein jensen	8	(100/8)8	(13/12)92.3	12
-Both	8	100 (8/8)	(13/13)100	13

Table 2 shows Recovery rates of *M tuberculosis* by BACTEC MGIT 960 compared with LJ medium. Out of

the 21 *M tuberculosis* isolated by both system, 21 were recovered in BACTEC MGIT 960 ; the recovery rate was 100% (21/ 21). Whereas 20 were recovered on L J medium; the recovery rate of *M tuberculosis* was 95.2% (20/21). The recovery rates for positive and negative smears are revealed in table 2.

Mean no. days (range) to detection of <i>M tuberculosis</i> complex				
Media	All specimens	Smear positive	Smear negative	x p
BACTEC MGIT 960	10.1(6-12)	9.0(6-11.9)	(12-7)10.9	41.0 0.001>
Lowenstein Jensen	23.7(14-56)	(21-14)18.4	(56-14)26.8	

$X^2 \geq 3.84$, $p < 0.05$ (significant).

The time to detection (TTD) of *M tuberculosis* by BACTEC MGIT 960 and Lowenstein Jensen medium was shown in Table 3. For all specimens, the mean (range) time to detection of *M tuberculosis* was 10.1(6-12) days in the BACTEC MGIT 960. On LJ medium, the mean (range) time was 23.7(14-56) days. For smear-positive specimens, the mean (range) time to detection was 9.0(6-11) in the BACTEC MGIT 960. On LJ medium, the mean (range) time for smear-positive specimens was 18.4(14-21). Whereas in smear-negative specimens, the mean (range) time to detection was (10.9(7-12) days for *M. tuberculosis* in BACTEC MGIT 960. Whereas on LJ medium, the mean (range) time for smear-negative specimens was 26.8(14-56) days.. The difference in TTD between BACTEC MGIT 960 and LJ for *M. tuberculosis* was statistically highly significant ($x = 41.0$, $p = 0.001$).

Discussion

Tuberculosis (TB) is responsible for about one third of preventable deaths worldwide (16). Rapid diagnosis of *Mycobacterium tuberculosis* infection is critical; therefore, attempts to shorten the time needed for detection of such pathogen deserve attention. BACTEC MGIT 960 system

was used in this study as a rapid method for detecting *M tuberculosis* compared with L J medium. Of the total 150 sputum specimen collected from patients suspected for tuberculosis and examined, 21 (14%) were positive for *M tuberculosis* by both BACTEC MGIT 960 instrument and L J medium, of which 8 (38.10%) were AFB smear positive and 13 (61.90 %) were AFB smear negative . The result of this study slightly agreed with that reported by Somoskövi et al (14.6%) (17) . As a single method. 21 *M tuberculosis* were detected in BACTEC MGIT 960 system, whereas 20 were detected on L J medium. It is possible that the one isolate detected by BACTEC MGIT 960 alone did not metabolize the [14C]palmitic acid in BACTEC 12B or that the higher volume of the 7-ml BACTEC MGIT 960 diluted potential growth inhibitors in the specimen(17). . Each system detected all 8 smear-positive specimens. Contamination did not cause a serious problem in our study, where the rates of contamination were 6 (4.00%) and 4 (4.66%) for BACTEC MGIT 960 and LJ medium respectively .

Regarding rates of recovery, in the present study, the automated BACTEC MGIT 960 system displayed a rate of recovery of *M. tuberculosis* ((100% (21/ 21))). Whereas the recovery rate of *M tuberculosis* on Lowenstein Jensen was 95.2% (20/21) which was higher than those previously reported 88% by Tortoli et al.(18) and 77% by Hanna et al. (19) . The lower rate of recovery observed by Hanna et al. and Tortoli et al. may have been due to a higher contamination rate with BACTEC MGIT 960. Hanna et al. found that after removal of the contaminated cohorts from their analysis, the sensitivity of BACTEC MGIT 960 increased from 77 to 86%(19) .

The time to detection (TTD) for *M.tuberculosis* with BACTEC MGIT 960 (10.1 days) was much faster than that on LJ (23.7 days) with statically significant difference ($x^2 = 41.0$, $p < 0.001$). The TTD in this work for *M. tuberculosis* (10.1 days) with the BACTEC MGIT960 was shorter than that reported by Tazawa et al.(20) (13.5days) , Kobayashi et al. (14.1 days) (21) . In contrast , it was longer

than the time of 7.2 days observed by Somoskovi and Magyar(22), and slightly longer than that reported by Rishiet al (9.66 days)(23) and Pyffer et al (9.9 days) (24). Considering the different TTD for smear-positive and smear-negative specimens, which may be expected to have fewer bacilli in the originating specimen, and consequently a longer TTD, it was compared the TTD of *M. tuberculosis* from smear-positive and smear-negative specimens. As may be expected, the TTD of the BACTEC MGIT 960 for smear-positive specimens (9.0 days) was shorter than that of smear-negative specimens (10.9 days). In conclusion, the BACTEC MGIT 960 system is a rapid more suitable method for detection of *M tuberculosis* and that is characterized by detection time that is shorter than that of the “gold standard (Lowenstein Jensen medium) and more sensitive. It also has the additional advantages of being a fully automated, high capacity, non-radiometric and non-invasive system (24).

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