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ORIGINAL ARTICLE

COMPARISON BETWEEN MYCOBACTERIA GROWTH INDICATOR TUBE (MGIT), BACTEC 460 TB SYSTEM AND LOWENSTEIN-JENSEN MEDIUM FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS

By

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Background: *Diagnosis of sputum negative pulmonary tuberculosis is a major health problem. So, the emergence of new techniques for more precise and rapid microbiological identification of mycobacterium tuberculosis in clinical samples is of great importance to improve the tuberculosis management outcome worldwide.* **Patient and Methods:** *Sputum and or BAL samples were collected from 150 patients of suspected pulmonary TB, all were cultured on solid medium (Lowenstein-Jensen) and on liquid media (BACTEC 460 and MGIT).*

Results: *Out of the 150 samples, only 30 were culture positive. The positive specimens for L-J media, BACTEC 460, and MGIT were 16 (53.3%), 25 (83.3%), and 24 (80%) respectively. There was a statistical significance (P<0.05) between both liquid media and L-J media. By combining 2 medias together, the culture positive combinations were 28 (93.3%) for L-J + MGIT (combination A), 28 (93.3%) for L-J + BACTEC 460 (combination B), and 29 (96.6%) for BACTEC 460 + MGIT (combination C), with no statistical difference between them. The time to detection (TTD) was highly statistically significant between both liquid media and L-J media (P<0.01).*

Conclusion: *The use of liquid media (BACTEC 460 and MGIT) is more accurate and rapid method for diagnosis of smear negative pulmonary tuberculosis, the combination of more than one media is highly recommended for rapid and precise diagnosis.*

Keywords: *Tuberculosis, Rapid detection, BACTEC, MGIT.*

INTRODUCTION

More than eight million people develop active TB annually, and approximately two million die from the disease each year. The WHO estimate that there are more than 15 million people living with TB. In 2003, out of estimation 8.8 million new TB cases worldwide, 3.9 million were diagnosed by laboratory testing and 674,000 also were HIV positive. An estimated 1.7 million people died of TB in 2003, 22 % of whom were co-infected with HIV. Those with active TB who receive no treatment can infect an average of 10 to 15 people annually. Although TB is curable, it kills 5000 people every day, 98% of deaths are in developing world affecting mostly young adults in their most productive year.(1)

So tuberculosis remains a major health threat, and the rapid emergence of drug resistant mycobacteria has strengthed the demand for rapid methods for detection of

mycobacteria in clinical samples. Since the prevention of tuberculosis relies on the early detection and cure the infectious cases. So current efforts are focused upon improving the rapidity of identification of M. tuberculosis (MBT), allowing prompt initiation of appropriate therapy.[2]

PATIENTS AND METHOD

This study was conducted on 150 patients suspected clinically and radiologically to have pulmonary tuberculosis who were admitted to Al Abbasia Chest Hospital between February 2006 and February 2007. A total of 150 specimens received from these patients in that interval were investigated including sputum (135 specimens) and bronchoalveolar lavage (BAL) (15 specimens). The sputum specimens were investigated by direct sputum smear stained with Ziehl-Neelsen (Z-N) for three successive days, they were sputum negative for AFB. The BAL specimens examined also by direct smear stained with Z-N, they were negative for AFB. Then we cultured the sputum or the bronchoalveolar lavage on Lowenstein-Jensen (L-J) medium, BACTEC 460 TB system and MGIT tube. Of these 150 specimens 30 specimens only were culture positive for MTB.

- ***All patients were subjected to:***

1. Full history taking.
2. Clinical examination.
3. Chest x-ray.
4. Complete blood picture.
5. SGOT, SGPT, Blood Urea, Serum creatinine
6. Sampling for microbiological study which may be sputum or broncho-alveolar lavage to be examined by:-
   a) Direct smear stained by Ziehl-Neelsen.
   b) Culture on Lowenstein-Jensen medium.
   c) Culture on BACTEC 460 TB system.
   d) Culture on mycobacteria growth indicator tube (MGIT).

- Culture on MGIT tube.

The MGIT culture tube contains 4 ml of Middlebrook 7H9 broth base, to which we added 0.5 ml of an enrichment supplement containing oleic acid, albumin, dextrose, and catalase (BBL MGIT OADC), and 0.1 ml of an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA). After inoculation of each tube with 0.5 ml of the processed specimen, the tubes were incubated at 37ºC. We read tubes daily starting on the second day of incubation using UV lamp.[2]

- Preparation of Interpretive Negative and Positive Control Tubes

Use of the Positive and Negative Control tubes is only for the interpretation of fluorescence and is not intended as a control for the performance of the media.

Positive Control tubes can be used many times. Each Positive Control tube can be used for up to four weeks when stored at room temperature.

Negative Control Tube: An unopened, uninoculated MGIT tube is used as a control.

RESULTS

One hundred and seventeen patients were males (78%); Thirty three patients were females (22%). The age of the patients ranged from 15 to 85 years. 135 patients were investigated by direct sputum smear stained with Ziehl-Neelsen for three successive days; they were sputum negative for acid fast bacilli (AFB). The bronchoalveolar lavage was obtained from 15 patients, of those 150 patients 30 patients were culture positive for MTB.

Of those culture positive patients, twenty-one patients were males (70%); nine patients were females (30%). The age of the patients ranged from 15 to 80 years with the mean age 36.5 years +/- 13.8 SD. In all, 30 specimens that were culture positive for MTB, 12 specimens (40%) were bronchoalveolar lavage (BAL), and 18 specimens (60%) were sputum (SP).

A comparison of MGIT tube with the BACTEC 460 TB system and L-J medium for detection of mycobacterium tuberculosis complex in 30 specimens is shown in Tables 1-4.

Table 1. Showing rates of recovery of mycobacteria tuberculosis complex from clinical specimens using solid and liquid cultures media.

Culture method	NO. (%) of isolates recovered
L-J	16 (53.3%)
BACTEC 460	25 (83.3%)
MGIT tube	24 (80%)
L-J + BACTEC 460	28 (93.3%)
L-J + MGIT tube	28 (93.3%)
BACTEC 460 + MGIT Tube	29 (96.6%)

Table 2. Comparison of recovery rate between L-J and MGIT tube.

Results	L-J	MGIT tube
Positive	16	24
Negative	14	6
X^2=4.8 P=o.o2	P<0.05.	

Table 3. Comparison of recovery rate between L-J and BACTEC460.

Results	L-J	BACTEC 460
Positive	16	25
Negative	14	5
X^2=6.2 P=0.012	P<0.05.	

Table 4. Comparison of recovery rate between BACTEC 460 MGIT tube.

Results	BACTEC460	MGIT tube
Positive	25	24
Negative	5	6
X^2=0.11 P=o.738	P>0.05.	

MGIT tube, BACTEC 460 TB system and L-J medium detected 80%, 83.3% and 53.3% respectively of M. tuberculosis complex isolates (Fig 1). These indicate that the 2 liquid media (MGIT tube and BACTEC 460) were significantly more sensitive than solid medium (L-J) [MGIT versus L-J, P < 0.05 shown in Table 2; BACTEC versus L-J, P < 0.05 shown in Table 3 but there was no statistical significance between MGIT and BACTEC 460 (P > 0.05) shown in Table 4.

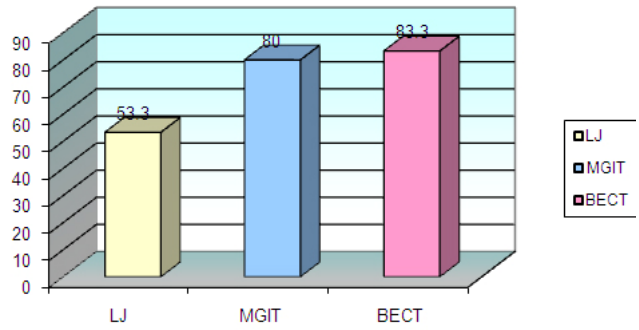

Fig 1. Showing the recovery rates of the three culture media

By comparing the combinations of more than one method of culture which are: Combination A (MGIT + L-J media), Combination B (BACTEC 460 + L-J media), and Combination C (MGIT + BACTEC 460) the following results were obtained as shown in Tables 5-7.

Table 5. Comparison of recovery rates between combination A,B.

Results	Combination A	Combination B
Positive	28	28
Negative	2	2
X^2=0.00 P=1.000	P>0.05.	

Table 6. Comparison of recovery rate between combination A,C.

Results	Combination A	Combination C
Positive	28	29
Negative	2	1
X^2=0.35 P=0.55	P>0.05.	

Table 7. Comparison of recovery rate between combination B,C.

Results	Combination B	Combination C
Positive	28	29
Negative	2	1
X^2=0.35 P=0.55	P>0.05.	

When comparing the recovery rates on liquid and solid media in combination (gold standard), MGIT plus L-J (combination A) recovered 28 of 30 M. tuberculosis complex isolates (93.3%), BACTEC 460 plus L-J (Combination B) also recovered 28 of 30 MBT complex isolates (93.3%). There was no statistically significant difference (P>0.05) between the two gold standards, Table 5.

The two mycobacterial broths MGIT plus BACTEC460 (combination C) yielded 29 of 30 M. tuberculosis complex isolates (96.6%). No statistically significant was found between combination A and C (P>0.05) Table (6) or between combination B and C (P>0.05) Table (7) for the recovery of MBT complex.

Comparing the results of results between either solid media (L-J) and liquid media (BACTEC 460 or MGIT) and combination A, or B, the following results were obtained in Tables 8-11.

Table 8. Comparison of recovery rate between combination A and L-J.

Result	L-J	Combination A
Positive	16	28
Negative	14	2
X^2=12.2 P=0.00045 P<0.01.		

Table 9. Comparison of recovery rate between Combination A and MGIT.

Result	MGIT tube	Combination A
Positive	24	28
Negative	4	2
X^2=2.3 P=0.128 P>0.05.		

Table 10. Comparison of recovery rate between combination B and L-J.

Results	L-J	Combination B
Positive	16	28
Negative	14	2
X^2=12.2 P=0.00045 P<0.01.		

Table 11. Comparison between combination B and BACTEC460.

Results	BACTEC 460	Combination B
Positive	25	28
Negative	5	2
X^2=1.46 P=0.22 P>0.05.		

When comparing each combination with each single media of its component separately, we found that there was highly statistically significant (P < 0.01) between combination A (L-J +MGIT) recovered 28 (93.3%) of 30 MTB isolates versus L-J recovered 16 (53.3%) of 30 MTB isolates Table 8, while there was no statistical significance (P>0.05) between combination A and MGIT tube recovered 24 0f 30 (80.3%) MTB isolates Table 9. There was highly statistically significant (P<0.01) between combination B (L-J + BACTEC 460) recovered 28 of 30 (93.3%) MTB isolates versus L-J medium recovered 16 of 30 (53.3%) of MTB isolates Table (10), while there was no statistically significant (P>0.05) between combination B and BACTEC 460 recovered 25 of 30 (83.3%) MTB isolates Table 11.

Comparing each of the liquid media alone (MGIT and BACTEC 460), and the combination of both (combination C), the following results were obtained in Tables 12,13.

Table 12. Comparison between combination C and MGIT tube.

Results	MGIT tube	Combination C
Positive	24	29
Negative	6	1
X^2=4.04 P=0.044 P<0.05.		

Table 13. Comparison between combination C and BACTEC 460.

Results	BACTEC460	Combination C
Positive	25	29
Negative	5	1
X^2=2.96 P=0.08 P>0.05.		

There was statistically significant (P<0.05) between combination C (MGIT + BACTEC460) recovered 29 of 30 (96.6%) MTB isolates versus MGIT recovered 24 of 30 (80%) MTB isolates Table 12, while there was no statistically significant (P>0.05) between combination C and BACTEC 460 recovered 25 of 30 (83.3%) MTB isolates Table 13.

Many isolates grew only on a single medium, while they didn't grow on any other ones. MGIT tube detected 2 isolates of M. tuberculosis which were missed by BACTEC 460 and L-J medium, while BACTEC 460 detected also 2 isolates of M. tuberculosis which were missed by MGIT tube and L-J medium. Solid media finally detected 1 isolate of M. tuberculosis which was missed by MGIT tube and BACTEC 460.

Table 14. Showing the mean TTD of MTB for L-J, BACTEC460, and MGIT.

	Group (1) L.J culture	Group (2) BACTEC460	Group (3) MGIT tube	f-test	
Mean (days)	31 ± 9.4	12 ± 4	18.4 ± 14.2	27.49	P = 0.00 P <0.01 HS

In Table 14, the means and the ranges of times to detection (TTD) of positive cultures were 31 +/- 9.4 SD (range 22- 47) days for L-J , 12 +/- 4 SD (range 5-34) days for BACTEC 460 and 18.4 +/- 14.2 (range 5-57) days for MGIT tube.

Table 15. Comparison between the mean TTD for the 3 cultures media.

Group (1), (2)	Group (1), (3)	Group (2), (3)
t-test= 103.7	t-test= 16.42	t-test= 5.6
P=0.000	P=0.0001	P=0.020
P<0.01 HS	P<0.01 HS	P<0.05 S

The mean time to detection was shorter for BACTEC 460 than MGIT tube, and the difference was statistically significant (P<0.05). The mean TTD was shorter for MGIT than L-J, and the difference was highly statistically significant (P<0.01). Finally the mean TTD was shorter for BACTEC 460 than L-J, and also the difference was highly statistically significant (P<0.01), Table 15.

The earliest Growth of MTB was detected in the MGIT after 5 days, similar to what has been observed for BACTEC460 (after 5 days also). In contrast, the earliest growth of MTB on L-J was not observed before 22 days. MGIT beyond 34 days (the maximum incubation period for BACTEC460 in our study) resulted in detection of 4 additional isolates 2 of them were negative on both BACTEC460 and L-J, 1 of them was –ve on BACTEC 460 and the last one was positive on both BACTEC and L-J. MGIT beyond 47 days (Maximum incubation period for L-J in our study) resulted in detection of 3 additional isolates two of them were negative on both BACTEC460 and L-J.

The contamination rate was 6.6% for MGIT, 0% for both BACTEC460 and L-J medium respectively.

DISCUSSION

The aim of this work was to evaluate the mycobacteria growth indicator tube (MGIT) for the detection of MTB in the sputum or the BAL of patients suspected clinically and radiologically to have pulmonary tuberculosis with negative direct sputum smear stained with Ziehl-Neelsen for three successive days. Then the results were compared with those of the reference BACTEC 460 TB system and of Lowenstein-Jensen solid medium in term of recovery rate and mean time to detection.

The smears negative pulmonary TB were focused on because these patients are also capable of transmitting the infection. The relative transmission rate of smear-negative TB patients compared to smear-positive TB patients has been calculated at 22% using a molecular epidemiologic technique. Although persons with smear-negative TB are less infectious than the smear-positive patients, their overall contribution to disease transmission is considerable because half of all patients with TB can present with negative sputum smear findings (from 8.8 million cases diagnosed in 2002, 3.9 million cases only were sputum positive according to WHO report 2004). Despite the initial clinical suspicion of TB, when a patient's sputum smear results are negative for AFB, the diagnosis of TB may be missed. For those patients with a high clinical suspicion, clinicians must face the dilemma of empirically treating or waiting for up to 8 weeks for the final culture results.[3]

In this study, the rates of recovery of MTB were 80% (24 of 30) for MGIT tube, 83.3% (25 of 30) for BACTEC 460 and 53.3% (16 of 30) for L-J medium. The MGIT tube and BACTEC 460 sensitivities were not significantly different (P>0.05), and both showed significantly higher sensitivity than did L-J (P< 0.05).

This agrees with Badak et al[4] who compared the MGIT tube with the BACTEC TB-460 and LJ culture medium and found that The cultures sensitivities from smear negative specimens were 75.3% (64 of 85) for MGIT tube, 72.9% (62 of 85) for BACTEC 460, and 58.8% (50 of 85) for L-J medium. They also found that the MGIT tube and BACTEC sensitivities were not statistically different overall, and both showed significantly higher sensitivity than did L-J which completely agrees with results obtained in this study.

But the result achieved in this study for MGIT tube is higher than that obtained by Pfyffer et al[5] who found the rates of recovery of mycobacteria in smear negative specimens were 68.2% (75 of 110) for MGIT tube, 82.7% (91 of 110) for BACTEC460, and 59.1% (65 of 110) for L-J. The authors found that MGIT sensitivity was higher than that of solid media but the difference was statistically insignificant (P>0.05) while the sensitivity of BACTEC 460 was higher than MGIT and the difference was also statistically significant (P<0.05), finally the sensitivity of BACTEC460 versus solid media showed highly statistical significance (P<0.01).

The sensitivity of MGIT tube was 68.2% in the study of Pfyffer et al[5] which was lower than the result obtained in this study because the samples were collected from 3 centers, which may result in a delay in sample processing (2-5 days after specimen collection), which may affect the viability of the bacilli found in the specimen. Also contamination ranged from 2%, 6.1%, to 13.8% between the three centers. While in this study the sample processing occurred in the day of collection and the contamination rate for MGIT tube was 6.6%.

The sensitivity of MGIT tube in this study was lower than that reported by Somoskovi et al[6] who found that the recovery rate of MTB from the smear negative specimens was 95.1% for BACTEC MGIT 960. This can be explained by using of the automated BACTEC MGIT 960 TB system. But the sensitivity of MGIT tube in this study was higher than that reported by Huang et al (2) who found that the recovery rate of MTB from the smear negative specimens was 67% for BACTEC MGIT 960 which can be explained by the high contamination rate occurred with Huang et al 2001.

The sensitivities of MGIT, BACTEC 460, and L-J medium were higher when the cultures were done from the smear positive specimens. In the study of Badak et al[4] culture sensitivities from smear positive specimens were 94.6%, 95.7%, and 93.5% for MGIT, BACTEC460, and L-J medium respectively. And Pfyffer et al[5] found that the sensitivities

were 88.6%, 95.7% and 85.7% for MGIT, BACTEC460, and L-J medium respectively. This may be due to the large number of the bacilli present in the smear positive specimens while the number of the bacilli was much smaller in smear negative specimens. The higher sensitivity of MGIT tube reported by other studies (7 and 8) who reported sensitivity of 100% can be explained by using the smear positive specimens (about 93% of all specimens)[7] on the other hand the low sensitivity of MGIT 67.5% reported by El-Shinawy et al[9] can be explained by low sensitivity of direct sputum smear examination (80% positive) and also he selected 40 cases of pulmonary TB diagnosed be PCR.

It is generally accepted that the use of a combination of liquid plus solid media (gold standard) is essential in good laboratory for the isolation of mycobacteria.[5]

The results obtained in this study demonstrated that there was no statistically significant difference between a gold standard consisting of MGIT plus L-J medium (combination A) or BACTEC 460 plus L-J medium (combination B) for the recovery of M. tuberculosis isolates (93.3% versus 93.3%, respectively; P>0.05). The combination of 2 liquid media MGIT plus BACTEC460 (combination C) was, however, even more efficient in isolation of mycobacteria than the use of gold standards described above (combination A, 93.3%; combination B, 93.3%; combination C, 96.6%) there were no statistical significance between the three combinations. This result was in agreement with that reported by Pfyffer et al[5] who reported no statistically significant difference between the three combinations for MTB isolates. However there were statistically significant differences between combination A and C for the total number of mycobacterial isolates and non-tuberculous mycobacteria.

The use of L-J medium in combination with one of both liquid media (MGIT or BACTEC460) increased the yield to 93.3% for each of them and increases the sensitivity by 13.3% for MGIT and by 10% for BACTEC460. Badak et al[4] reported that the use of L-J in combination with MGIT or BACTEC460 increased the sensitivity by 7% to 8% of each of them which is comparable with the results obtained in this study. Moreover, the mycobacteria detection rates were significantly higher than when L-J used alone. The sensitivity of L-J increased from 53.3% to 93.3% when used in combination with either MGIT or BACTEC460. This finding suggests that the L-J should not be used alone. This finding agrees with that reported by Huang et al.[2]

Many isolates grew only on a single medium, while they didn't grow on any other ones. MGIT tube detected 2 isolates of M. tuberculosis which were missed by BACTEC 460 and L-J medium, while BACTEC 460 detected also 2 isolates of M. tuberculosis which were missed by MGIT tube and L-J medium. Solid medium finally detected 1 isolate of M. tuberculosis which was missed by MGIT tube and BACTEC 460. The culture failures of the three systems in recovering of mycobacterium from the 30 specimens were due to smear negativity of all specimens included in this study. These results are in agreement with that reported by Badak et al.[2]

In this study, the mean times to detection (TTD) of mycobacteria in smear negative patients were 18.4, 12, 31 days in MGIT, in BACTEC460, and on L-J medium respectively. The mean time to detection was shorter for BACTEC 460 than MGIT tube, and the difference was statistically significant (P<0.05). The TTD was shorter for MGIT than L-J, and the difference was highly statistically significant (P<0.01). Finally the TTD was shorter for BACTEC 460 than L-J, and also the difference was highly statistically significant (P<0.01). This results are in agreement with that reported by Pfyffer et al[5] as the mean times to detection for smear negative specimens were 20.3, 18, 27.2 days in MGIT, in BACTEC460, and on L-J medium respectively, but there was insignificant difference in mean TTD between MGIT and BACTEC460, while the difference was significant in this study due to small number of patients included in it.

In this study, the mean times to detection of mycobacteria disagree with that reported by Huang et al[2] who stated that the mean TTD for smear negative specimens were 14.9, and 19.3 days for MGIT 960 and BACTEC 460 respectively (P<0.05) this can be explained by using of the automated BACTEC 460 and MGIT 960 systems which can read the tubes every 60 minutes and can detect the fluorescence earlier than naked eye with the UV lamp in manual MGIT. Also the mean time to detection (TTD) for MGIT tube in this study was longer than that either reported by Somoskovi et al[6] who found that TTD of MTB from smear negative specimens for BACTEC MGIT 960 was 15.8 days or by Chien et al[10] who found that TTD of MTB from smear negative specimens was 16.5 days.

Regarding to smear positive specimens the mean TTD of mycobacteria for MGIT was 9.9 days as reported by Pfyffer et al,[5] 3.3 days as reported by Kamel et al[8] and 5 days as reported by Sheble et al.[11] The marked shortening in the mean TTD of mycobacteria for MGIT with smear positive specimens can be explained by the large number of bacilli present in these specimens.

The contamination rate was not a serious problem in this study, 6.6% for MGIT, 0% for both BACTEC460 and L-J medium respectively. In the study of Badak et al[4] it was 5.6% for MGIT, 3.9% for BACTEC460, and 6.7% for L-J, which was nearly the same for MGIT but the zero contamination rates for both BACTEC460 and L-J in this study were due to our small sample number of patients included in it and the strict decontamination policy.

In this study, the sensitivity and the time to detection of

MGIT were comparable to those of BACTEC 460, although MGIT require longer time to detect mycobacteria from smear negative specimens. The 2 liquid media (MGIT tube and BACTEC 460) were significantly more sensitive than solid medium (L-J), the mean TTD for both liquid media were shorter than that of L-J and the difference was highly significant. This is in agreement with Badak et al.[4]

The combination of liquid and solid media remains the best. MGIT tube can be considered an excellent replacement for BACTEC460 in the cultural gold standard (BACTEC460 +L-J), since no statistically significant difference between combination A (MGIT +L-J) and combination B (BACTEC460+L-J). Moreover, the mycobacteria detection rates were significantly higher than when L-J used alone, so we suggest that L-J should not be used alone. This is in agreement with Huang et al.[2]

The combination of both liquid media, combination C (MGIT+BACTEC460), however, is more efficient in isolation of mycobacteria than the use of gold standards (combination A, 93.3%; combination B, 93.3%; combination C, 96.6%) there were no statistical significance between the three combinations. But the usage of combination C is limited by the cost and the disadvantages of radioactive material within BACTEC460. This is in agreement with Pfyffer et al.[5]

BACTEC 460 TB system uses a radiometric method for the detection of mycobacterial growth. Disposal of radioactive waste produced by this system presents a problem and increases the costs.[6] The usage of radioactive material is not found with MGIT giving it the superiority.

From all of the above, the rapidity and the sensitivity by which the mycobacteria can be detected by MGIT tube make it suitable nonradiometric alternative to BACTEC460 and the usage of automated BACTEC 960 TB system make MGIT tube more rapid and more sensitive. This is in agreement with Somoskovi et al.[6]

The MGIT system is simple and easy to use non-radiometric system and could prove to be a cost effective alternative for more rapid isolation of mycobacteria in laboratories, compared with the highly expensive BACTEC 460 TB system.[12]

The BACTEC MGIT 960 TB system has the advantages that it is easy to use and has shorter detection time, higher capacity, and provides fully automated, continuous monitoring for mycobacteria from clinical samples, which are all important consideration in selecting a microbiology system.[2]

The BACTEC 960/MGIT shows new and interesting features, such as a shorter time to detection of acid-fast bacilli and more convenient technology. These favorable features, coupled with an elevated diagnostic accuracy on almost all the clinically most important mycobacterial species, make the BACTEC 960/MGIT system in combination with conventional solid media a valuable alternative to the radiometric system.[13]

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