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Recovery of Mycobacteria from Clinical Specimens and Assessing Drug Susceptibility Test of Mycobacterium Tuberculosis Specimens by Mycobacteria Growth Indicator Tube (MGIT)

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ABSTRACT

Background: Tuberculosis is a disease of global importance. Indeed, the lack of sensitive methods for the diagnosis and inappropriate therapy may lead to increased multidrug-resistance (MDR) cases. However, early detection and identification of acid fast bacilli (AFB) in clinical specimens can lead to effective intervention.

Materials and Methods: The sputum specimens from 156 clinically suspected tuberculosis patients and 40 non- tuberculosis patients were digested, examined microscopically for acid- fast bacilli, and inoculated into "Mycobacterium Growth Indicator Tube" (MGIT), BACTEC –12B vial and onto Lowenstein- Jensen slants by standard procedures.

Results: The result showed that smear was positive in 82(52.5%) and negative in 74 (47.5%) of 156 clinically suspected tuberculosis patients. The culture positive rate with Lowenstein- Jensen, MGIT, and BACTEC-12B vial were 122(78%), 136(87%), and 143(91%), respectively. Thereafter, MGIT indirect and direct susceptibility tests were performed on 15 sputum-positive specimens and the results were compared with proportional method. The results have revealed that accordance with proportional method was higher in MGIT indirect (83.5%) than direct (75%) susceptibility test, the difference was significant ($p < 0.05$). In another set of experiments, the indirect MGIT drug susceptibility test in 25 mycobacterium tuberculosis isolates were performed and compared with proportional method. The results showed that MGIT could correctly detect susceptibility to streptomycin, ethambutol, rifampin and isoniazid for 77.8%, 33%, 77.2% and 80%, respectively. Also, the agreement with proportional method for resistance were 88% for streptomycin, 80% for ethambutol, 80% for rifampin and 89% for isoniazid. Furthermore, by combining MGIT technology with L.J media, the mean time required for culture to grow for identification test was reduced from 22-28 to 12-16 days ($p < 0.05$).

Conclusion: MGIT is an efficient system to be used in center/ referral mycobacteriology laboratories of developing countries along with routine solid or liquid culture media. (Tanaffos 2002; 1(3): 35-44)

Key words: Mycobacterium tuberculosis, MGIT (Mycobacterium Growth Indicator Tube), Drug susceptibility test, Clinical specimen.

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INTRODUCTION

Tuberculosis is a disease of global importance. One third of the world population is estimated to be infected with mycobacterium tuberculosis and eight million new cases of tuberculosis arise each year (1). The most powerful weapons for control of tuberculosis are case finding and proper treatment (2). Indeed, the lack of sensitive methods for the diagnosis and inappropriate therapy may lead to increased multidrug-resistance (MDR) cases, increased opportunities for spread of the disease in community, and increased mortality (3,4,5). However, early detection and identification of acid-fast bacilli (AFB) in clinical specimens can lead to effective intervention (6). Recent publications from Center for Disease Control (CDC) and Prevention (Atlanta, GA) have recommended turnaround times extending up to 21 days for isolation and identification of mycobacterium tuberculosis (7). Laboratories should aim not only at achieving this goal, but also at using a combination of solid and liquid media, which is the current “gold standard” for cultural detection of mycobacterium tuberculosis (8,9).

Introduction of the liquid-medium-based BACTEC-460 TB radiometric System (Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD) has been the hallmark for rapid detection of mycobacterium tuberculosis complex (10,11). The main limitations of the system are the high cost, disposal of the radioactive waste and the need for instrumentation. The Mycobacteria Growth Indicator Tube (MGIT) (Becton-Dickinson Sparks, MD, USA) was introduced as a nonradiometric alternative to the BACTEC 460 TB system for rapid growth and detection of mycobacteria (12). In the earlier studies it has been demonstrated that MGIT detects growth of AFB from clinical specimens with a high degree of accuracy and as rapidly as BACTEC 460 system(13).

In the present study, we have primarily aimed to evaluate the rate of recovery and the required time to detect mycobacterium tuberculosis from clinical specimens by MGIT system in comparison with radiometric and solid L.J medium. Meanwhile, we analyzed the possible relation between number of bacilli/ml in the inoculum and the required number of days for detection of positive signal in MGIT and other media.

Prior investigators have suggested that the MGIT system may also be used for testing susceptibility of mycobacterium tuberculosis isolates (14). They have mostly compared the MGIT susceptibility test with radiometric or proportional method against isoniazid and rifampin (15,16). We have compared the susceptibility test of MGIT with proportional method against all the four primary drugs; streptomycin (SM), ethambutol (EMB), isoniazid (INH), and rifampin (RF). Since results from direct susceptibility test are available much faster than those from indirect test, we tried to determine whether there is any difference between results of direct and indirect MGIT susceptibility test as compared with the proportional method using the same clinical specimens. We also evaluated the performance of the combined MGIT technology with L. J culture media for identification of mycobacterium tuberculosis in clinical specimens.

MATERIALS AND METHODS

Clinical specimens

Sputum specimens were collected from 156 tuberculosis patients referred or admitted to “National Research Institute of Tuberculosis and Lung Disease (NRITLD)” for diagnosis of tuberculosis from August 1999 to February 2000. Another 40 sputum specimens were collected from patients who had disease other than mycobacterial infection and were considered as negative controls.

Specimen processing

Specimens were digested and decontaminated by N-acety-L-Cystein NaOH method, as described by Kent and Kubica with a final NaOH concentration of 1%. After decontamination, smears were prepared from the concentrated sediments of the specimens for Ziehl- Neelsen (ZN) acid-fast staining (17).

Inoculation and cultivation of clinical specimens

One-half milliliter of the processed specimen was inoculated into MGIT tubes with the MGIT growth supplement added to it, 0.5ml was inoculated into BACTEC-12B vial, and 0.2 ml was inoculated onto each of four L.J slants (prepared in our laboratory). All inoculated media were incubated at 37⁰C for 8 weeks. MGIT tubes were examined daily with 365-nm UV light (using UV transilluminator) for 8 weeks. Any tubes that showed fluorescence comparable to that of positive chemical control (a 0.4% sodium sulfite solution inoculated into MGIT tube) were considered positive. If no fluorescence was seen after 8 weeks, the MGIT tube was regarded as negative.

The BACTEC 12B vials were examined for growth index (GI) twice per week for the first 2 weeks and weekly thereafter for an additional 6 weeks. (The time to detection in the BACTEC system was set at the interval between specimen inoculation and growth index of >50 in 12B vial). The inoculated solid L.J media were inspected weekly for 8 weeks. All positive results verified by Ziehl-Neelsen staining. We routinely inoculated four L.J medium tubes for each specimen. However, for the comparative analysis only one L.J tube (first inoculated), one 12B vial and one MGIT tube were taken into consideration.

Susceptibility test by MGIT

MGIT susceptibility test was performed according to the manufacturer's recommendations (11,12). For each isolate tested, 5 tubes were prepared: four of the tubes contained the anti-tuberculosis drugs, and one

was a drug-free growth control. To all tubes, 0.5ml of MGIT OADC (oleic acid, bovine serum albumin, dextrose, and catalase) growth supplement was added, and 0.1ml of the antibiotic solution was added to each drug-containing tube, give the final concentration of 0.8µg/ml of streptomycin, 0.1µg/ml of isoniazid, 1.0µg/ml of rifampin, and 3.5µg/ml of ethambutol.

All 5 tubes were inoculated with 0.5ml of the 1:5 diluted suspension of an isolate. For the indirect susceptibility testing, 25 previous clinical isolates of *M. tuberculosis* were used. These stock cultures were first grown on solid culture medium. Colonies were removed with loop from surface of L.J medium and suspended in Middle Brook 7H9 Broth. Turbidity of suspension was adjusted to 0.5 McFarland. According to manufacturer's instructions, 1ml of suspension was diluted with 4ml of sterile saline (1:5 dilution), 0.5 ml of diluted suspension was inoculated into labeled MGIT tubes. If the results of MGIT direct susceptibility testing were different from the results obtained by proportional method, (which was considered as the reference method), both assays would be repeated.

In another set of experiment, direct susceptibility test was performed on 15 smear-positive sputum specimens. Having the specimen processed, the sediment was used to inoculate into MGIT drug susceptibility set as described earlier. These specimens were also evaluated by indirect susceptibility test once the primary isolation MGIT tubes become positive for mycobacterium tuberculosis growth. 1.0 ml of suspension from MGIT positive tube was subcultured into a fresh MGIT tube and kept at 37⁰C. Once the tube was positive, it was vortexed and 1.0 ml of the suspension was diluted into 4 ml of sterile saline (1:5). This 1:5 diluted suspension was used to inoculate 0.5 ml into each of the labeled MGIT tubes.

Susceptibility test by proportional method

Colonies from surface of L.J medium were transferred into sterile test tube containing 6-8 glass beads and 3.0 ml of Middle Brook 7H9 Broth. The suspension was adjusted to 1 McFarland standard. Thereafter, The dilutions of 10^{-1} , 10^{-3} , 10^{-5} were prepared and inoculated into drug-containing media and controls.

Routine identification test

Identification tests were performed using MGIT and L.J combination only from 30 selected TB patients admitted to our institute. Once the MGIT tubes showed a positive signal, which was much earlier than growth on L.J slants, a protein of broth was centrifuged and 0.2ml of sediment was inoculated into fresh L.J medium and incubated at 37°C. Centrifugation was carried out to expedite growth on L.J. Once the colonies were in sufficient number, the identification tests (such as niacin, nitrate and catalase) were performed.

RESULTS

Comparison of MGIT system with radiometric and L.J medium

A total of 156 patients were studied who were suspected of having tuberculosis (TB) according to their chest x-ray and clinical symptoms. Of 156 specimens, 82(52.5%) were smear positive and 74(47.5%) were smear negative. (Table 1)

Table 1. Rates of recovery of mycobacteria from clinical specimens using different culture media.

	Microscopy findings	Media used for culture		
		L.J	MGIT	BACTEC
clinically suspected patients (n=156)	Positive Smear (n=82)	63(+) 17(-) 2*	72 (+) 6(-) 4*	73 (+) 6(-) 3*
	Negative Smear (n=74)	59(+) 10(-) 5*	64(+) 4(-) 6*	70(+) 2(-) 2*

* contaminated

Of 156 specimens, BACTEC-12B recovered 143(91%) of all mycobacteria, while MGIT and L.J yielded 136(87%) and 122(78%) isolates, respectively.

All control subjects were revealed to be smear negative, of whom 37(92.5%) were L.J culture-negative and the remaining 3 culture slants were excluded due to contamination. Meanwhile, in control subjects the cultures were negative in 35(87.5%) and 36(90%) specimens by MGIT and BACTEC, respectively. Five MGIT tubes and 4 BACTEC-12B vials were contaminated. Thereby, the overall contamination rates for BACTEC, MGIT, and L.J media were 4.6, 7.6, and 5.1%, respectively.

The sensitivity and specificity of BACTEC-12B were 87.1 and 90%, respectively. L.J showed the highest specificity (93%), but the lowest sensitivity (60%). The sensitivity and specificity of MGIT were 81.9 and 89%, respectively.

Mean time of positive results:

The average number of days required for the detection of positive signal by each culture system is detailed in table 2.

Table 2. Range of time (days) required for detection of mycobacterium tuberculosis in clinical specimen with different culture media

Microscopic findings(n=156)	Culture method (days)		
	MGIT*	BACTEC**	L.J***
(3+) smear (n=32)	2-3	10-15	22-28
(2+) smear (n=20)	2-3	10-15	22-28
(1+) smear (n=30)	4-5	10-15	22-28
Few bacilli smear (n=54)	7-12	10-15	28-42
Negative smear (n=20)	7-12	10-15	28-42

* examined daily with 365-nm UV light for 8 weeks

** examined twice per week for the first 2 weeks and weekly for an additional 6 weeks

*** examined weekly for 8 weeks

The mean times to detection were 6.6 (2-12), 11.7 (10-15), and 27 days (20-24) with MGIT, BACTEC, and L.J, respectively ($p < 0.05$). In addition, our

experiment demonstrated a direct relationship between number of bacilli in processed specimen and the number of days to detection of positive results in MGIT tube. Thereby, in specimens with microscopy result of 3+, the MGIT tubes required only 2-3 days to show a positive signal, whereas in specimens with negative microscopy results, about 7-12 days were required ($p < 0.05$).

Comparison of indirect MGIT susceptibility test with proportional method

In every experimental setup, ATCC control strains ($H_{37}R_v$) performed as expected with streptomycin. Initial results showed an agreement for 19 (76%) out of 25 isolates by both method. After repeating the test with streptomycin the disagreement in the remaining 6 isolates were resolved and agreement by both methods was 22 out of 25 isolates. Thereby, the overall agreement by both methods to SM was 82% (Table 3).

Table 3. Comparison of MGIT susceptibility test results with proportional method (25 stock culture)

Drug	Both-S	Both-R	MGIT-R MOP-S	MGIT-S MOP-R	Percent of Agreement
SM	11	8	5 (3)*	1	76
INH	11	8	5 (3)	1	76
RF	11	7	4 (1)	3 (2)	72
ETB	12	8	3 (1)	2	80

* Number of disagreement(s) resolved after testing, are shown in parenthesis.

Initial ethambutol results obtained by both methods agreed for 20 (80%) isolates (12 isolates were susceptible and 8 were resistant). After repeating the tests, the disagreement resolved in one isolate and ethambutol results agreed for 21 (84%) out of 25 isolates. The overall agreement was 82%.

Results with the same clinical isolates revealed that the overall agreement by both methods for RF and INH were 78% and 82%, respectively. The mean time from inoculation to days at which result could be interpreted was 5.6 days (3-9 days) for MGIT, compared to a mean of 31 days (26-42 days) in proportional methods ($p < 0.001$).

Directed and indirect MGIT susceptibility test

Direct MGIT susceptibility test against streptomycin agreed with proportional method for 11(73%) out of 15 isolates (Table 4); however, with indirect MGIT susceptibility test 13(87%) out of 15 were agreed. In the same way with ethambutol and rifampin susceptibility results by direct MGIT test were agreed with proportional method for 12 (80%) and 10(67%), respectively. However, once the test was performed by indirect MGIT susceptibility test the agreement rose to 13 (87%), and 12(80%) out of 15 isolates against ethambutol and rifampin, respectively.

The susceptibility against isoniazid with MGIT direct and indirect test was both 12(80%) out of 15 isolates. The agreement of indirect MGIT susceptibility test with proportional method was higher (83.5%) than the direct MGIT susceptibility (75%), and the difference was statistically significant ($p < 0.05$).

MGIT combined with L.J media for performing routine identification test

Having the processed sputum specimens inoculated into L.J culture slant, the average of 20-28 days was required for culture to grow. Thereafter, we performed identification by routine biochemical test (niacin, nitrate and catalase). The time required for isolation and identification of clinical isolates was 31-35 days.

On the other hand, when the same clinical specimens inoculated first into MGIT and after detecting positive signal, 0.2ml of its sediment was inoculated into fresh L.J medium; at this time, the required time for colony formation was only 12-16 days.

In this way, we were able to reduce the average time required for isolation and identification to 12 days. The difference was statistically significant ($p < 0.05$).

Table 4. Comparison of MGIT direct and indirect susceptibility test with the method of proportion.

Isolate No	Result of susceptibility by MGIT direct test (direct specimens inoculation)				Result of susceptibility by MGIT indirect test (colonies inoculation)				Conventional proportional method			
	SM	RF	ETB	INH	SM	RF	ETB	INH	SM	RF	ETB	INH
1) IR 280	S	R	S	S	S	R	S	S	S	S	S	S
2) IR 301	R	R	R	R	S	R	R	R	R	R	R	R
3) IR 218	S	R	R	S	R	R	S	S	R	R	S	S
4) IR 105	S	S	S	R	S	S	S	R	S	S	S	S
5) IR 107	S	R	S	R	S	R	S	R	S	S	S	S
6) IR 16	S	R	S	R	R	R	S	R	R	R	R	R
7) IR 56	R	R	R	R	R	R	S	R	R	R	S	R
8) IR 30	R	R	S	S	R	S	R	S	S	S	S	S
9) IR 112	S	S	S	S	S	S	S	S	S	S	S	S
10) IR 308	R	R	R	R	R	R	R	R	R	S	R	S
11) IR 402	S	R	S	R	S	R	S	R	S	R	S	R
12) IR 589	S	R	S	R	S	R	S	R	S	R	S	R
13) IR 4	S	S	S	S	S	S	S	S	S	S	S	S
14) IR 462	R	R	R	R	R	R	R	R	R	R	R	R
15) IR 297	S	S	S	R	S	R	R	R	R	R	R	R

DISCUSSION

Rapid-diagnosis of tuberculosis is important in the control and prevention of the disease. Today, in most of the developing countries the tuberculosis diagnosis still depends on isolation of *M. tuberculosis* by solid culture medium (L.J) which takes up 6 to 8 weeks (9,17,18). Thereby, for fast and efficient diagnosis strategies, the new technology should be applied in clinical mycobacteriology laboratories. Recently, the non-radiometric mycobacteria growth indicator tube (MGIT) was introduced for rapid growth and detection of mycobacteria (12,14,15,16). The MGIT consists of a 16 (by 100 mm round) bottom tube containing an enriched 7H9 broth. Embedded in silicon at the bottom of the tube is a fluorescent indicator that is quenched in the presence of oxygen. When bacteria are actively growing inside the tube, they consume oxygen presenting in the broth, and the indicator becomes fluorescent when exposed to 365-nm light. In the present study, we have

compared MGIT with established cultivation technology, L.J media and radiometric BACTEC-12B TB system. Results have revealed that the rate of recovery in smear-positive cases were more or less similar between BACTEC-12B and MGIT culture media, while the recovery in L.J medium was less than the two liquid media systems. The difference was significant ($p < 0.05$).

The recovery of mycobacterium tuberculosis in smear-negative cases was slightly lower in MGIT system (86%) versus BACTEC (94%); however, in L.J culture medium it was even lower (80%) ($p < 0.05$). In a similar study, Pfyffer et al. demonstrated that the difference in isolation of mycobacterium tuberculosis from smear-positive specimen by MGIT and solid culture media were statistically significant, meanwhile, the difference in smear-negative cases with MGIT and L.J was significant, too ($p < 0.05$) (11). In another study, Sion

et al. demonstrated that MGIT system could recover more mycobacteria from clinical specimen than solid culture media (19). Our findings are more or less the same as those of other investigators (13,18,19) who have stated that the recovery rate was highest in BACTEC followed by MGIT and L.J culture medium. However, if we combine results of all the four L.J tubes inoculated per specimen routinely in our laboratory, the performance of L.J medium improves significantly and the recovery rate would become as good as the other two media. Hence, we may increase the chance of recovery by using more L.J culture media in comparison with one culture tube used by MGIT/BACTEC system. The greatest advantage is the rapid reporting results by liquid system. The mean time of mycobacterium tuberculosis detection by MGIT was 6.8 days versus 11.7 and 27 days by BACTEC-12B and L.J media, respectively ($p < 0.05$). Therefore, the rapidity and non-radiometric component of the system can be the most obvious advantages of MGIT against BACTEC and L.J media.

Contamination was not a serious problem. The contamination rate with MGIT was 7.1% versus 5.1% for L.J and BACTEC-12B, which was not statistically significant and was within the accepted range. The contaminated microorganisms isolated were staphylococcus and streptococcus. The most likely explanation for slightly higher contamination in MGIT is the richness of its medium compared to the other media. These values are quite comparable with those reported by Palaci et al. (20) (7.8% for MGIT and 4.4% for L.J medium) and Cornfield et al. (21) (12.2% for MGIT and 5.5% for BACTEC system).

It is known that clinical laboratories can play critical roles in the control of tuberculosis through timely detection, species identification, and drug susceptibility testing to ensure adequate and appropriate treatment. Delays in the diagnosis of

tuberculosis seriously impact both patients and tuberculosis control program. Therefore, particular attention must be given to usefulness of any new cultivation system for its capability to isolate and perform susceptibility test. Thus, we evaluated the performance of susceptibility test by MGIT and compared the result with proportional method. Totally, the correlation of MGIT susceptibility results with method of proportional for SM, ETB, RF, and INH was 82%, 82%, 78%, and 82%, respectively. In a similar study, Bergmann and Woods showed MGIT and method of proportional results agreed for 93% and 90% against SM and ETB, respectively (14).

In our study, the percentage of agreement between MGIT and proportional method was less than previous reports (14,15,16), indeed, the number of isolates was only 25 along with 2 ATCC standard strains; thus, additional studies of the MGIT system for susceptibility testing required to draw any conclusion.

Since, direct susceptibility test in certain situation may save significant time in reporting susceptibility test results, we tried this approach with MGIT. We also tested the same specimen by indirect susceptibility after isolation of the culture and compared the two results with proportional method. We observed that the agreement with proportional method was higher in the indirect than direct susceptibility MGIT test and the difference was statistically significant ($p < 0.05$). Nevertheless, the short time of results interpretation in direct MGIT susceptibility test would be counted as most important advantage over MGIT indirect susceptibility test and proportional method. Based on the results, we conclude that in some situations it would be reasonable to perform direct susceptibility test and confirm it later with indirect test (table 4).

We noticed that the high cost of MGIT in comparison with L.J medium might prevent its

routine use in most of intermediate laboratories of Iran. However, it is recommended that Iranian regional/reference and central laboratories should standardize their testing methods and incorporate new and rapid techniques. In this study, we have demonstrated that by combining MGIT technology with L.J medium, one can recover almost all culture-positive specimens, especially those that are smear-negative. The mean-time required for reporting a positive culture is significantly shorter. A complete report of isolation, identification and susceptibility testing can be obtained within 30 days as recommended by CDC (8).

In summary, our results indicate that MGIT is an efficient system to be used in central/reference mycobacteriology laboratories along with routine solid culture media. However, with usual workload, and due to the high cost of the liquid media and reagents, it may not be feasible to use this system unless additional financial resources are available.

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