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Original Article

Comparison of the performance of TK system with LJ and MGIT methods in the diagnosis of tuberculosis

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Abstract: Tuberculosis is a common infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* (TB). Various liquid or solid media are used for the diagnosis of tuberculosis. TK Rapid Mycobacterial Culture System has been developed recently. In our study, we aimed to compare TK Rapid Mycobacterial Culture System with LJ and MGIT systems in the diagnosis of tuberculosis. 200 clinical specimens (152 sputum, 41 Bronchoalveolar lavage fluid (BAL), 4 gastric aspirations, 2 urine and 1 wound) obtained from 192 patients from different clinics were included for the diagnosis of TB. All specimens were decontaminated by using the same-common procedure in all the methods. The obtained sediment was used for inoculation for the BACTEC MGIT 960, TK and LJ. Additionally, smears were prepared from the residual suspension for Ehrlich-Ziehl-Neelsen (EZN) staining for microscopic examination. Contamination was observed in 23 sputum and 4 BAL samples. Contamination rates for TK, LJ, and BACTEC MGIT 960 systems were determined as 3 (1.5%), 13 (6.5%), and 18 (9%) respectively. *Mycobacterium tuberculosis* growth was determined as 15 (7.5%), 14 (7%) and 13 (6.5%) by TK culture system, MGIT and LJ, respectively. In our study, the total mean detection times of *Mycobacterium tuberculosis* by the LJ, TK, and MGIT method were 20.1, 17.1, and 8.3 days, respectively. TK system showed a dramatically lower contamination rate than the others. There was no difference in growth rates for each of the three methods. We concluded that the TK culture system is disadvantageous in terms of turnaround time.

Keywords: *Mycobacterium tuberculosis,* TK system, BACTEC MGIT 960 system

Introduction

Tuberculosis, is a common infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. It has been known since prehistoric times, and is as old as humanity. Tuberculosis typically affects the lungs, but can also spread to other parts of the body. *Mycobacterium tuberculosis* is located in almost every part of nature, such as waters, pastures, soil, sludge, and air. Tuberculosis spreads mainly through the respiratory tract. Cell wall of *Mycobacterium tuberculosis* bacilli is thicker compared to other bacteria and provides a high lipid [1]. Due to these circumstances, its growth and illness mechanism is unique. Therefore, special methods are required for its isolation and identification. Acid-fast staining methods are used for the diagnosis of tuberculosis in various clinical samples, and predictive value is >90% for *M. tuberculosis*. However, the sensitivity of staining methods is low. On the other hand, culture methods provide isolates for identification and drug susceptibility testing, but the delay in obtaining results remains a disadvantage. Over the past years, several new techniques have been developed for a rapid diagnosis; however, culture methods are still the gold standard [2].

Various liquid or solid media are used for the diagnosis of tuberculosis. Löwenstein-Jensen (LJ) is used commonly, and this traditional solid medium still plays a role in the recovery of mycobacteria from clinical samples. Petragnani, Middlebrook 7H10 and Middlebrook 7H11 are other examples for solid media. On the other hand, 7H9 and Dubos Tween-albumin liquid medium are used basically for preparation of inoculum for subcultures and antibiotic susceptibility tests. Today, broth-based automatic systems could provide a rapid result and have

been in use for many years. BACTEC 460TB System (BD Diagnostic Systems), MGIT 960 System (BD Diagnostic Systems), MB/BacT System (BioMerieux, Durham, NC), BACTEC MYCO/F Lytic blood culture bottle (BD Diagnostic Systems), ESP Culture System II (Trek Diagnostic) and TK Culture System (Salubris Inc,) can be given as examples [3, 4].

The BACTEC MGIT 960 is an automated system that exploits the fluorescence of an oxygen sensor to detect growth of mycobacteria in culture. It is specially designed to accommodate Mycobacteria Growth Indicator Tube (MGIT) [5].

TK Rapid Mycobacterial Culture System has been developed recently. The system uses TK media, which is a ready-to-use, rapid mycobacterial culture medium. TK media is incubated and read by Mycolor TK automated incubator reader [6-8]. The system promises some advantages. In case of growth of mycobacteria, TK media will change color from red to yellow whereas in the presence of contaminant microorganisms, formation of the color green is observed. TK is a solid-based medium and this situation is especially a handicap and a disadvantage for susceptibility tests. Already, CDC (Centers for Disease Control and Prevention) recommends that Laboratories performing TB cultures should routinely use a broth based system [9]. Therefore, TK broth media has been available from the manufacturer recently. There are a limited number of studies on the performance and advantages of TK tuberculosis culture system in daily use in a diagnostic laboratory.

TK liquid media and Mycolor TK should be tested for performance and advantages in the diagnosis of tuberculosis by comparison with LJ and MGIT systems, which have been used for many years. Our study was conducted for this purpose.

Material and methods

This study was carried out between May and August 2012 in the Mycobacteriology Laboratory of the Department of Microbiology and Clinical Microbiology, Meram Medical school. The identification systems used in our laboratory were validated by an external quality control program. The analytical sensitivity of our systems is 101-102 bacilli/ml of sample for the diagnostic yield. MGIT requires two to four weeks for the growth of M. tuberculosis and LJ requires four to seven weeks for the growth of M. tuberculosis.

200 clinical specimens obtained from 192 patients from different clinics were included in the diagnosis of TB. Among them, there were 152 sputum, 41 BAL, 4 gastric aspirations, 2 urine and 1 wound samples.

All the specimens were processed using the modified Petroff's method with double the volume of NaOH (4%) for sputa and equal volume of NaOH (2%) for extrapulmonary specimens. The specimens were shaken for homogenization and then decontaminated. Finally, distilled water was added to the mixture and mixed in a centrifuge (3000G) for 15 minutes. The obtained sediment was used for inoculation for all the three methods. Additionally, smears were prepared from the residual suspension for EZN staining for microscopic examination. The amounts inoculated for the BACTEC MGIT 960, TK and LJ were 0.5 ml, 0.1 ml and 0.1 ml, respectively. In addition, OADC (oleic acid, bovine serum albumin, dextrose, catalase) and PANTA (polymyxin, amphotericin B, nalidixic acid, trimethoprim, azlocillin) were added to MGIT tubes. LJ tubes were incubated in a 37°C incubator (in 5-10% CO_2), MGIT tubes were incubated in BACTEC MGIT 960 instrument and TK Media were observed by automated incubator reader Mycolor TK. The incubation periods for MGIT system, LJ and TK system were 8 weeks. The growth detection time for MGIT tubes and TK Media were recorded as indicated by BACTEC MGIT 960 and Mycolor TK system. LJ tubes were checked for mycobacterial colonies every other day. The growth detection time was recorded for each of the three culture methods. Statistical analysis of mycobacterial isolation and contamination rates was done using McNemar test ($p<0.0001$) and comparative time-to-growth detection by t test ($p< 0.001$).

Results

A total of 200 specimens were decontaminated by using the same common procedure in all the three methods. Contamination was determined in at least one of the methods among the 26 samples. Contamination was observed in 23 sputum and 4 BAL samples. Contamination

Table 1. The comparative contamination rates

Patient No	Specimen	LJ	MGIT	TK
1	Sputum	-	+	-
15	Sputum	-	+	-
23	Sputum	-	-	+
34	Sputum	+	+	-
36	Sputum	-	+	-
47	BAL	+	-	-
49	Sputum	+	-	-
50	Sputum	-	+	-
63	Sputum	-	+	-
76	Sputum	+	-	-
77	Sputum	-	+	-
85	BAL	+	-	-
86	Sputum	+	+	-
94	Sputum	+	+	-
95	Sputum	-	-	+
103	Sputum	+	+	-
109	Sputum	-	+	-
112	Sputum	+	+	-
119	BAL	-	+	-
123	Sputum	+	-	-
137	BAL	-	+	-
140	Sputum	-	+	-
158	Sputum	-	+	-
159	Sputum	+	-	-
161	Sputum	-	-	+
167	Sputum	+	+	-
192	Sputum	+	+	-
Total number (percent)		13 (6.5)	18 (9)	3 (1.5)*

*(X^2=6.25) ($p<0.0001$).

rates for LJ and TK were determined as 13 (6.5%) and 3 (1.5%), respectively (**Table 1**). On the other hand, the highest number of contaminant bacteria were isolated in the BACTEC MGIT 960 system with 18 (9%) samples. There was no difference in terms of the contamination rate between LJ and MGIT, but the contamination rate for TK was different from that obtained by LJ and MGIT. This difference was statistically significant ($p<0.0001$).

14 (7%) samples were identified as positive in smear by the EZN staining and growth was determined in all of these samples by all three methods.

The growth of Mycobacteria in any of the methods was considered significant. Mycobacteria were isolated from 15 samples and the results were positive by TK culture system in all of them (7.5%). Mycobacterium growth was determined as 14 (7%) and 13 (6.5%) by MGIT and LJ, respectively (**Table 2**).

In our study, the total mean detection times of mycobacteria by the LJ, TK, and MGIT method were 20.1, 17.1, and 8.3 days, respectively. This difference was statistically significant ($p<0.0001$) (**Table 2**).

Discussion

In this study, 200 clinical specimens obtained from 192 patients from different clinics were evaluated. The bacilli were detected in 14 (7%) samples by EZN Staining whereas they were identified in 15 (7.5%) specimens by culture methods. Our laboratory is the busiest Tuberculosis diagnostic laboratory of this region and has the highest capacity at the same time. The samples were sent to our laboratory from different clinics and regions. The rates are considered reasonable according to the above facts and the information in the relevant literature. Bacilli detection rate was found to be 6.2% in a recent study in our country [10]. However, this rate was reported as 27% in a similar study [11]. The reason for this is that the research was conducted in a hospital for chest diseases and therefore it may be more likely a case of tuberculosis. In our study, 15 positive results were obtained with the TK system whereas the positive culture results were determined as 14 (7%) and 13 (6.5%) by MGIT and LJ, respectively. However, this isn't considered to be a significant difference. Some smear-positive samples yielded negative results with MGIT and LJ. This is undesirable for these methods. So, there is a risk of false negative results in routine diagnostic laboratories for these methods.

Contamination is an undesirable situation and it should be minimized. In our study, contamination rates for MGIT, LJ and TK were determined as 18 (9%), 13 (6.5%) and 3 (1.5%), respectively. TK system showed a dramatically lower contamination rate compared with the others. Moreover, TK Medium does not contain selective antimicrobials. Considering that the same common decontamination protocol was implemented, it may have been caused by the form of tubes and caps or the selectivity of the content of the medium. In addition, a different

Table 2. The comparative results

Patient No	Specimen	AFB	Time To Growth Detection (days)		
			LJ	MGIT	TK
2	BAL	+	16	6	12
7	BAL	+	24	12	21
22	Sputum	++++	21	6	19
33	Sputum	+	27	10	29
42	BAL	+	13	6	10
49	Sputum	+	-	14	22
66	Sputum	+++	11	4	8
80	Sputum	+++	17	8	14
130	Sputum	++	19	8	15
134	Sputum	+	17	7	12
137	BAL	+	29	-	24
151	Sputum	+++	20	5	13
152	Sputum	+++	30	13	23
176	BAL	-	-	11	26
189	Sputum	++	17	6	8
Total number (percent)		14 (7)	13 (6.5)	14 (7)	15 (7.5)
(Median: days)			20.1*	8.3*	17.1*

*(p<0.001).

color (green) appears when contaminant bacteria grow in TK medium. It is a quick and an easy method. Also, MGIT requires preparatory work before inoculation of samples. This may have increased the likelihood of contamination for MGIT. In addition, the major inoculum used for the MGIT may have led to a higher contamination rate. In our opinion, it may be that higher inoculum is a risk factor for contamination and a disadvantage for this system.

The evaluation of growth detection time provided important information. Median growth detection times for MGIT, TK and LJ were 8.3, 17.1, and 20.1 days, respectively. MGIT system showed a significant difference compared to the other two methods. Similarly, in a recent study, it was found that the median times for positivity were 7 and 25 days for colorimetric methods, MGIT and LJ, respectively [11]. However, another research has recently noted that TK detection time of growth was close to the MGIT system [12]. The reason for this difference may be the usage of TK liquid medium in our study, whereas previous researchers had used TK solid medium.

Although it takes a long time to detect growth in a classical culture medium, culture method is very valuable in the diagnosis of tuberculosis. Researchs in this field aim to improve both faster and more reliable methods. In our study, we arrived at the conclusion that the MGIT system is advantageous in terms of turnaround time. However, we thought that some cases might be missed, because the result was negative in one smear-positive sample. Also, contamination rates were dramatically higher than the other methods. When we were planning this work, we particularly wondered what the performance of the TK liquid medium and TK system would be because, though few in number, there were studies regarding solid medium in the literature. TK liquid medium, which is a recently developed and Mycolor automated system, showed a very good performance in terms of the isolation and contamination rates. It is important for a reliable diagnosis of tuberculosis. However, time to growth detection of TK system is significantly longer than the MGIT system. Contrary to our results, in another study where LJ, MGIT and TK systems were compared, the isolation rate of MGIT method was determined as the highest and the most contamination was identified in the TK solid medium. The performance of TK liquid medium may have given rise to this difference. TK system provides growth results earlier than LJ classical culture media, it is ready for use, mycobacterial growth and contamination can be distinguished by visual and the contamination rates are satisfactory. But still, time to growth detection is far behind MGIT system. TK culture system is promising in the diagnosis of tuberculosis and future studies can provide new information about it.

Disclosure of conflict of interest

None.

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References

[1] In: Murray PR, Rosenthal KS, Pfaller MA, editors. Medical microbiolog. 5th edition. Phila-

delphia, USA: Elsevier Mosby, 2005; pp: 297-301.
[2] Özkara Ş, Kılıçaslan Z. TORAKS books–Tuberculosis. 11th edition. AVES publishing, 2010; pp: 92-110.
[3] Huang TS, Chen CS, Lee SS, Huang WK, Liu YC. Comparison of the BACTEC MGIT 960 and BACTEC 460TB systems for detection of mycobacteria in clinical specimens. Ann Clin Lab Sci 2001; 31: 279-83.
[4] Baylan O. Culture based diagnostic methods for tuberculosis. Mikrobiyol Bul 2005; 39: 107-24.
[5] Hanna BA, Ebrahimzadeh A, Elliott LB, Morgan MA, Novak SM, Rusch-Gerdes S, Acio M, Dunbar DF, Holmes TM, Rexer CH, Savthyakumar C, Vannier AM. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. J Clin Microbiol 1999; 37: 748-52.
[6] Baylan O, Kisa O, Albay A, Doganci L. Evaluation of a new automated, rapid, colorimetric culture system using solid medium for laboratory diagnosis of tuberculosis and determination of anti-tuberculosis drug susceptibility. Int J Tuberc Lung Dis 2004; 8: 772-7.
[7] Akyar I, Kocagoz T, Sinik G, Oktem S, Aytekin N, Kocagoz S. Lateral flow assay for rapid differentiation of Mycobacterium tuberculosis complex and 97 species of mycobacteria other than tuberculosis grown in Löwenstein-Jensen and TK-SLC medium. Indian J Med Microbiol 2010; 28: 308-12.
[8] Kocagöz T, Altın S, Türkyılmaz Ö, Taş I, Karaduman P, Bolaban D, Yeşilyurt E, Öktem S, Aytekin N, Şınık G, Mozioğlu E, Silier T. Efficiency of the TK Culture System in the diagnosis of tuberculosis. Diagn Microbiol Infect Dis 2012; 72: 350-7.
[9] To View or Order the Core Curriculum on Tuberculosis. Sixth Edition 2013. Available at: http://www.cdc.gov/tb/education/corecurr/pdf/introduction.pdf. Accessed May 26, 2013.
[10] Sesli Cetin E, Aynali A, Öztürk T, Özseven AG, Kaya S. Evaluation of the Bactec Mycobacteria Growth Indicator Tube 960 system with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. SDÜ Tıp Fak Derg 2012; 19: 12-16.
[11] Boum Y 2nd, Orikiriza P, Rojas-Ponce G, Riera-Montes M, Atwine D, Nansumba M, Bazira J, Tuyakira E, De Beaudrap P, Bonnet M, Page AL. Use of colorimetric culture methods for detection of Mycobacterium tuberculosis complex from sputum samples in resource-limited settings. J Clin Microbiol 2013; 51: 2273-9.
[12] Kocagöz T, Tiryaki S, Silier T, Güney C. TK SYSTEM The Colorimetric Mycobacterial Culture System Enables Rapid, Easy and Effective Diagnosis of Tuberculosis. Toronto, Canada: American Society for Microbiology, General Meeting 2007; pp: 21-25.