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ORIGINAL ARTICLE

Detection of Mycobacterium isolates with different methods and their resistance ratios against anti-tuberculosis drugs

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ABSTRACT

Objectives: The aim of the present study was to evaluate the efficacy (recovery rate, time to detection and Drug Susceptibility Tests –DST- of Mycobacteria-only B460) of new colorimetric medium, Dio-TK and to compare it with routinely used conventional media, Lowenstein Jensen (LJ) and Bactec 460 TB culture system.

Materials and methods: Totally 901 clinic specimens were investigated for assignment of tuberculosis by Ehrlich-Ziehl-Nielsen smear strain method, Lowenstein-Jensen, BACTEC 460TB and Dio-TK medium culture systems.

Results: Nineteen of 901 clinic specimens (2.1%) were positive by any of these methods. 17 (89.5%) of these specimens positive found by smear strain method, 17 (89.5%) by Lowenstein-Jensen, 19 (100%) by BACTEC 460TB and 14 (73.7%) by Dio-TK medium. NAP and Niacin identification tests were applied to *Mycobacterium strains*. 12 (63.1%) of 19 isolates were identified as *M.tuberculosis* complex and 7 (36.9%) were identified as Mycobacterium other than tuberculosis (MOTT) bacilli. 10 (83.3%) of 12 *M.tuberculosis* complex strains were not resistant to any major drug. But one of 2 isolate was resistant to streptomycin and the other one isolate was resistant to both streptomycin and isoniazid.

Conclusion: Our data suggest that some advantages (such as an early detection and differentiation mycobacterium growth from contamination) of the Dio-TK CS over other mycobacterial culture systems make it a practical and rapid system for daily use, and a suitable alternative to other currently available solid media, such as LJ, for detection time of mycobacteria and DST. *J Microbiol Infect Dis 2011;1 (1) :5-9.*

Key words: Dio-TK medium, *Mycobacterium tuberculosis*, detection, resistance

Mikobakteri izolatlarının farklı yöntemlerle saptanması ve antitüberküloz ilaçlara karşı direnç oranları

ÖZET

Amaç: Bu çalışmanın amacı, yeni bir kolorimetrik medium olan Dio-TK besiyerinin etkinliğinin (Mikobakterilerin saptanma oranı, deteksiyon zamanı ve İlaç Duyarlılık Testleri-DST) rutin olarak kullanılan geleneksel yöntemlerden Löwenstein Jensen (LJ) ve BACTEC 460 TB kültür sistemleri ile karşılaştırmaktır.

Gereç ve yöntem: Toplam 901 klinik örnekte Erlich Ziehl Nielsen (EZN) boyama yöntemi, Löwenstein-Jensen, BACTEC 460TB ve Dio-TK medium kültür sistemleri ile tüberkülozun belirlenmesi araştırılmıştır.

Bulgular: Toplam 901 klinik örneğin 19'u (%2.1) Mycobacterium açısından pozitif bulunmuştur. EZN boyama yöntemi ve LJ metodu ile bu örneklerin 17'si (%89.5) pozitif bulunurken, örneklerin 19'u (%100) BACTEC 460TB ve 14 (%73.7) Dio-TK medium ile pozitif saptanmıştır. Mycobacterium suşları için NAP ve Niasin tanımlama testleri uygulanmış, 19 izolatın 12'si (%63.1) *M.tuberculosis* kompleksi ve 7'si de (%36.9) tüberküloz basili dışındaki Mycobacteriumlar (MOTT) olarak tespit edilmiştir.[12] *M.tuberculosis* kompleks suşlarından 10'unda (%83.3) herhangi bir major antituberkuloz ilaca karşı dirençli saptanmamıştır. Diğer iki izolattan birisi streptomisine, diğeri ise ise Streptomisin ve isoniaside karşı dirençli bulunmuştur.

Sonuç: Bizim verilerimiz Dio-TK CS besiyerinin, mikobakterilerin saptanması ve DST için, LJ gibi günümüzde kullanılan diğer mikobakteriyel kültür sistemlerine göre saptama oranları ve süresi açısından bazı avantajlar sunması ile pratik ve hızlı bir sistem olarak günlük kullanım için uygun bir alternatif olabilecektir.

Anahtar kelimeler: Dio-TK medium, *Mycobacterium tuberculosis*, belirleme, direnç.

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INTRODUCTION

Tuberculosis (TB) is a significant public health problem for both industrialized and developing countries.[1] The increasing incidence of TB has made it essential for laboratories to quickly detect and identify mycobacteria from human clinical materials.[2] Cultural methods still represent the gold standard for the definitive diagnosis of TB and Drug Susceptibility Tests (DST) but delay in obtaining results remains as an important problem. Despite the advantages of broth-based culture systems, traditional solid media still play a role in recovering of mycobacteria from clinical samples and is recommended by the Centers for Disease Control and Prevention (CDC) for use along with liquid media.[2] The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* in many geographic areas and the increased migratory flux from higher-prevalence to lower-prevalence countries greater the importance of rapid identification and detection of drug resistance in optimal management of patients with TB.[1]

The BACTEC 460TB (B460) system (Becton Dickinson Biosciences, Sparks, Md.) has been widely validated for approximately 20 years and is regarded as the best method in clinical laboratories for reliable and rapid testing of susceptibility of *M.tuberculosis* isolates to front-line drugs such as streptomycin (SM), isoniazid (INH), rifampicin (RMP), ethambutol (EMB), and pyrazinamide (PZA), in accordance with the CDC recommendations. However, it is labor-intensive, bears the potential risk of cross-contamination, requires special attention regarding radioisotopes, and-as manufacture has been halted-will not be available any more in the future.[3,4]

Dio-TK Culture System (CS) (Salibrus Inc. Istanbul, Turkey) includes Dio TK Medium (TK Medium), Dio-TK SLC (selective), Dio-TK PNB (p-nitrobenzoic acid), Dio-TK INH (isoniazid), Dio-TK RMP (rifampicin), Dio-TK SM (streptomycin) and Dio-TK EMB (ethambutol) media. Dio TK is a rapid solid culture medium, with multiple color dye indicators that enables early detection of mycobacterium growth. During the incubation period, original red color of Dio-TK media turn to yellow with mycobacterium growth and to green with many other bacterial or fungal species. Dio-TK uses neither radioactivity nor ultraviolet light and it has an ability of differentiate mycobacterium growth from contamination.[2]

The aim of the present study was to evaluate the efficacy (recovery rate, time to detection and DST of Mycobacteria-only B460) of new colorimetric medium, Dio-TK and to compare it with routinely used conventional media, Lowenstein Jensen (LJ) and Bactec 460 TB culture system.

MATERIALS AND METHODS

Patients and specimens

In this study, 901 specimens, mostly sputum samples, obtained from 650 patients from different hospitals and clinics in Afyonkarahisar region were evaluated for the diagnosis of TB between March 2004 and February 2005 in the Mycobacteriology Laboratory of the department Of Medical Microbiology, Afyon Kocatepe University Hospital.

Culture procedures

A maximum of 10 ml of sputum and other viscous samples were collected into Dio-Safeprocess plastic 50 ml conical centrifuge tubes with glass beads. The same amount NaOH-NALC solution was added to tubes. After homogenization of all specimens by vortex, the tubes were incubated at room temperature or 10-15 min. After dilution with PBS (pH:6.8) to reach 50 ml solution in the tubes, each specimen was concentrated by refrigerated centrifugation at 4000x g. for 10-15 min. The supernatant was then discarded into emptied PBS tube, leaving the glass beads in the tube. The sediment remaining among the glass beads was vortexed to obtain a suspension for microscopy and culture. After processing of the sample, approximately half of the antiseptic solution was poured into the sample collection tube and the other half into the PBS tube; both tubes and their contents were discarded after 15 min. 100 µl suspension was inoculated into LJ solid medium, Dio-TK solid media and 100 µl was inoculated into BACTEC 12B liquid medium. All the media were incubated at 37°C in BACTEC 12B, LJ (in 5-10% CO_2) and Dio-TK medium for 30 days. Smears were prepared from the residual suspension by Ehrlich-Ziehl-Neelsen (EZN) staining for microscopic examination.

Culture of the samples by B460 and preparation of inoculums for DST were performed ac-

cording to the manufacturer's instruction.[9,10] If MTC growth was detected in the BACTEC 12B vials, DST was performed against to four first-line anti-tuberculosis drugs (INH, RMP, EMB and SM). The antibiotic concentration tested for EMB was 2.5 µg/ml, for INH 0.1 µg/ml, for SM 2.0 µg/ml and for RMP 2.0 µg/ml.[2] LJ Medium was examined once a week for 8 weeks. Smears of typical colonies on LJ medium were stained by EZN and examined microscopically. EZN staining was also used for confirmation of growth in Dio-TK media.

RESULTS

In this study, 901 samples (775 sputum, 67 Bronco-alveolar lavage fluid, 15 urine, 13 pleural fluid, 11 CSF, 2 peritoneal fluid, 11 gastric lavage, 3 abscess material and 4 synovial fluid) obtained from 650 patient (434 male, 216 female) were evaluated. The results of microscopic examination of stained smear were negative for 884 specimens (98.1%) and positive for 17 specimens (1.9%). Positive and negative results of B460 were compared with the results of EZN staining and positive, negative and contamination results of LJ, Dio-TK medium (Table 1).

Mycobacteria were isolated from 19 (100%), 17 (89.5%) and 14 (73.7%) of the specimens in Bactec 12B, LJ, Dio-TK Medium, respectively. One sample was smear positive but it is negative by all culture methods. Twelve samples were *M.tuberculosis* Complex (MTC) and seven were NTM on Bactec 12B. The contamination ratios for Bactec 12B, LJ and Dio-TK medium were 0.7% (n=7), 1.2% (n=11) and 1.7% (n=16), respectively.

The mean detection times of isolates by Bactec 12B (n=19), LJ (n=17) and Dio-TK Medium (n=14) were 11.7 (4-23), 25.4 (15-45) and 14.5 (6-23) days, respectively. The mean times to detect two isolates (grown Bactec 12B and LJ culture methods) without considering the Acid-Faste Bacilli (AFB) smear results, by B460 and LJ were 11.2 and 22.1 days, respectively.

DST of the MTC was performed using B460 (n=12). All the strains (n=12) were susceptible to EMB, RMP, two of these isolates were resistant to SM and one strain was resistant to both INH and SM by B460.

Table 1. Comparison of Bactec 12B results with EZN, LJ and Dio-TK medium results.

Samples (n=901)	EZN*		LJ**			DİO-TK		
	+	-	+	-	Cont	+	-	Cont
BACTEC (+) n:19	16	3	17	1	1	10	4	5
BACTEC (-) n:882	1	881	0	872	10	4	867	11

***EZN:** Ehrlich-Ziehl-Neelsen ****LJ:** Löwenstein Jensen Cont: contamination.

Table 2. Studies of Dio-TK CS reported by other authors.

	Patients (n)	Samples	AFP (+)	Mean Detection Time (days)		
				Bactec	LJ	Dio-TK medium
Kocagoz et al.[8] (n:50)	92	92	50	8.3	25.7	9.6
Ercis et al.[9] (n:15)	320	500	-	5	27	12
Bicmen et al.[10]	327	456	298	-	25	12.8
Baylan et al.[2] (n:13)	348	449	11	8.9	26.1	15.1
Present study (n:19)	650	901	17	11.7	25.4	14.5

DISCUSSION

Various different Media for culture of mycobacteria have been described, but only a few are in use today. The B460 culture system has gained a reputation as a standard for comparison with other newer systems.[6,7] The present study was undertaken to compare the newly developed automated Dio-TK medium against B460 (Table 2). In our study, the higher isolation rates for mycobacteria were detected by B460, following by LJ and Dio-TK medium, respectively. The number of positive mycobacteria samples was very low in our study; the rate of AFB true-positive samples was only 1.7% (n=16) in 901 samples. Some of the mycobacteria could only be isolated by using some of culture systems from the same inoculums, and thus different isolated rates were

obtained for different culture systems. This may be due to low numbers of mycobacteria in patient samples which resulted in insufficient inoculates in some of the media. In addition, the higher isolation rate B460 may be due to the procedure of repeat decontamination of contaminated cultures, which is note done for the other culture media.

Application of the same re-digestion procedure for LJ and Dio-TK media may also increase the isolation rate in these media.[2]

The CDC has recommended radiometric methods for DST because they can provide results more quickly than conventional testing solid media by first-line anti-tuberculosis drugs.[5] In the recent years, increasing numbers of multidrug-resistant strains of *M.tuberculosis* has stimulated efforts to develop rapid and accurate non-radiometric methods for DST.[2] Some limitation of Dio-TK media was observed by Baylan et al.[2] There were similar problems in our study. Dio-TK Media are pH sensitive, and if the NaOH treated samples are not properly neutralized, detection of growth by the color change from red to yellow takes longer. The risk of contamination is much greater during the inoculation procedure due to rubber stoppers using with the Dio-TK tubes compared to screw caps or vials. The color change from red to yellow may sometimes be due to Gram positive bacteria; any growth indication by color change should be confirmed by AFB staining to prevent false-positive results.[2]

While B460 detected mycobacteria most rapidly, Dio-TK was approximately two weeks faster than LJ medium. A comparison of Dio-TK CS and non-radioactive rapid mycobacterial culture systems would be very useful. As a conventional media such as LJ are most commonly used worldwide, although some of the disadvantages of Dio-TK CS may be a good alternative in these laboratories, saving about 2 weeks in primary isolation and ten days in DST.[11,12]

In recent years, molecular methods was commonly use of diagnosis of tuberculosis with speed, precision and quality.[13,14] Negi et al reported that PCR test sensitivity in all clinical samples were 72.7% and 75.9% respectively and found to be significantly higher when compared with those of other tests (ZN smear examination, LJ culture and BACTEC culture) . The mean detection time re-ported that for *M.tuberculosis* was 24.03 days by LJ medium culture, 12.89 days by BACTEC culture and less than one day by PCR test.[13]

In conclusion, our data suggest that despite its shortcomings, the some advantages (such as an early detection and differentiation mycobacterium growth from contamination) of the Dio-TK CS over other mycobacterial culture systems make it a practical and rapid system for daily use, and a suitable alternative to other currently available solid media, such as LJ, for detection time of mycobacteria and DST.[11]

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