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Comparison of the MGIT (Mycobacteria Growth Indicator Tube) with Ogawa media for recovery of Mycobacteria

Yeong Sic Kim, Yong Hyun Jo, Hee Joo Lee, Jin Tae Suh, and Young Ja Lee*

Department of Clinical Pathology, College of Medicine, Kyung Hee University, Seoul, Korea
Becton Dickinson Korea*

Background : It takes long time to cultivate *Mycobacterium tuberculosis* on solid media from clinical specimens. Although there is progress in the detection of tuberculosis using liquid media, Ogawa media is broadly used in Korea. In the 1990s, the BACTEC 460 system (Becton Dickinson, Sparks, MD, USA) was used in some laboratories in Korea, but at present, it is not used because of the accumulation of radioactive waste and the risk of cross-contamination. The BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA) is one of the new systems using liquid media. MGIT system uses oxygen-quenching fluorescence sensor technology instead of radioactive material. We evaluated MGIT for the sensitivity and specificity for the diagnosis of *Mycobacterium tuberculosis* by comparison with Ogawa media.

Methods : A total of 232 sputum specimens were collected from patients admitted to the hospital. All specimens were processed by 4% NaOH and 0.5% NALC. After inoculation of MGIT with 0.5 mL and Ogawa with 0.3 mL of the processed specimen, the media were observed every 3 days until 6 weeks and 8 weeks, respectively.

Results : A total of 99 isolates of mycobacteria were recovered from 232 specimens. Ninety nine isolates were detected with MGIT, as contrasted with 64 detected with Ogawa media. The mean times to detection of the *Mycobacterium* species were 12.6 days for MGIT, 23.7 days for Ogawa media. Contamination rates were 5.1% for MGIT, 5.6% for Ogawa media.

Conclusion : From our study, we conclude that MGIT is a superior method for recovery rate and time to detection of Mycobacteria to Ogawa media. *(Korean J Clin Microbiol 2001;4:58-61)*

Key words : MGIT, Ogawa, *Mycobacterium*

INTRODUCTION

It takes a long time to diagnose *Mycobacterium tuberculosis* because of the extended incubation time. Although Ogawa media is broadly used in Korea, it shows low sensitivity in detecting Mycobacteria[1]. As there is progress in the detection of Mycobacteria using liquid media, some laboratories in Korea used the BACTEC 460 TB system (Becton Dickinson, Sparks, MD, USA) in the 1990s. Because the BACTEC 460 TB system had problems, such as radioactive waste, a number of the new systems, such as the BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA), MB/BacT system (Organon Teknikia, Turnhout, Belgium), MB Redox (Biotest AG, Dreieich, Germany) have been developed[2-4]. The BACTEC MGIT 960 system uses oxygen-quenching fluorescence sensor technology instead of radioactive material. The large amount of dissolved oxygen quenches emissions of fluorescence from a fluorescent compound in MGIT. If the growth of Mycobacteria consumes the oxygen in MGIT, fluorescence in tube is emitted. We evaluated MGIT for the sensitivity and specificity for the diagnosis of Mycobacteria in comparison with Ogawa media.

접수번호 : CM 4-1-11
교 신 저 자 : 김영식
(130-702) 서울시 동대문구 회기동 1번지
경희의료원 임상병리과
Tel : 02-958-8674 Fax : 02-958-8609
E-mail: yeongsic@yahoo.co.kr

MATERIALS AND METHODS

Specimens: A total of 232 specimens, which were mainly

sputum from kyunghee university hospital patients suspected of pulmonary tuberculosis during september 1998, to november 1998, were collected. during september 1998 to november 1998. The processed specimen was inoculated on MGIT and Ogawa media.

1. AFB stain: Sputum without centrifugation was smeared and stained by Ziehl-Neelsen. When growth in MGIT and Ogawa media were seen, a smear was prepared using an AFB fixing agent and examined for AFB. The AFB fixing agent consisted of thimerosal 20 mg, phenol 0.5 mg, distilled water 80 mL, and rabbit serum 20 mL.

2. Inoculation on MGIT: Sputum was processed using the BBL MycoPrep™. Aseptically 0.5 mL of MGIT OADC and 0.1 mL of reconstituted MGIT PANTA antibiotic mixture in tube was added. One-half mL of the processed specimen was inoculated in the MGIT. MGIT was incubated at 37℃ and monitored every 3 days for 6 weeks using 365 nm UV transilluminator detecting fluorescence. If fluorescence was detected in the tube, the tube was considered positive and stained for AFB. Smear-AFB negative tube was checked for bacterial contamination.

3. Inoculation on Ogawa media: Sputum was processed using the BBL MycoPrep™. Inoculation of Ogawa media was done with 0.3 mL of the processed specimen. It was incubated at 37℃ and monitored every 3 days for 8 weeks. Colony on Ogawa media was stained for AFB. Smear-AFB negative was checked for bacterial contamination.

Table 1. Detection of *Mycobacterium* according to the initial AFB smear among culture positive specimens (99)

		Positive cultures in (No. of specimens)	
		MGIT	Ogawa
AFB positive	61	61	50
AFB negative	171	38	14
Total	232	99	64

RESULTS

In both culture systems, a total of 99 isolates were obtained from 232 specimens, of which 66 specimens showed positive for AFB stain (Table 1). Among culture-positive specimens all (99) were positive in MGIT, but only 64 specimens were positive on Ogawa media. MGIT detected 61 isolates of Mycobacteria from 61 smear-positive specimens and 38 isolates from 171 smear-negative specimens. But Ogawa media detected only 50 and 14 isolates, respectively. Overall, the mean time to detection for Mycobacteria was 12.6 days in MGIT and 23.7 days in Ogawa media (Table 2). The time to detection for Mycobacteria recovered in MGIT was 11.1 days faster than that of Ogawa media. From smear positive specimens in both culture systems, the number of AFB observed on smear was directly proportional to the time to detection, the faster time to detection was shown with 4+ smear-positive specimens (Table 3). Species identification for Mycobacteria was not performed. The contamination rate for 232 specimens noted with MGIT was 5.1%, while that with Ogawa media was 5.6%, overall.

DISCUSSION

There have been several studies on the detection of Mycobacteria by liquid media to evaluate the improvement of isolation and time to detection for Mycobacteria[5-7]. Although there were several reports that the BACTEC 460 TB system, worldwide used liquid media, is superior for recovery rate and time to detection to solid media, it had problems of cross contamination or radiactive waste[2,3,8-11]. The BACTEC MGIT 960 system is one among a number of new systems which is non-invasive and does not use radioactive materials. Hanna et al. reported that the BACTEC MGIT 960 system in combination with solid

Table 2. Time to detection of *Mycobacterium*

	Time to detection (days)		
	AFB negative	AFB positive	Total
MGIT (mean)	3-60(14.8)	5-35(11.3)	3-60(12.6)
Ogawa (mean)	6-35(17.6)	13-51(25.4)	6-51(23.7)

Table 3. Time to detection according to AFB stain

	Time to detection (days)				
	AFB -	AFB 1+	AFB 2+	AFB 3+	AFB 4+
MGIT (No. of specimens)	14.8 (38)	13.7 (19)	10.6 (23)	10.0 (18)	7.0 (1)
Ogawa (No. of specimens)	17.6 (14)	30.0 (12)	24.2 (21)	24.1 (16)	15.0 (1)

media showed performance comparable to that of the BACTEC 460 TB system for detection of *Mycobacterium tuberculosis* complex, while providing greater recovery of mycobacteria other than *M. tuberculosis* (MOTT)[2]. Pfyffer et al. also reported that MGIT proved to be a valuable alternative to the radiometric cultivation system[9]. Although we were not able to compare MGIT to the BACTEC 460 TB system, a comparison with Ogawa media was done and the effectiveness of MGIT was identified as compared to Ogawa media, which is widely used in this country. AFB positive specimens were culture positive in MGIT while Ogawa media cultivated only 50 specimens. Of 171 AFB negative specimens, 38 specimens were culture positive in MGIT, while Ogawa media cultivated only 14 specimens. This shows that the detection rate of MGIT is superior to that of the Ogawa media. The mean period of time required to detect AFB-positive growth using the MGIT was 11.1 days faster than that of the Ogawa media, and it had a significant statistical value ($p<0.01$). It should be taken into consideration the fact that 0.5 mL of the processed specimen was inoculated in MGIT, while only 0.3 mL in Ogawa media. With Ogawa media, the time needed to detect Mycobacteria in the 14 AFB smear negative specimens was shorter than that in the 50 AFB smear positive specimens, which needs further confirmation in accuracy by increasing the number of samples. If we were able to observe daily the emission of fluorescence in this study, we could have shortened the period necessary for detection of Mycobacteria. Also, automatic detection of emission of fluorescence with the BACTEC MGIT 960 system may have brought about a better and more accurate result. The identification of positive cultures was not performed in this study, so the difference in the time to detection between different species could not be identified. There was no difference of contamination rate between MGIT and Ogawa media. The contamination rate of MGIT and Ogawa media were 5.1% and 5.6%, repectively. When processing was performed according to our routine method (NaOH), the contamination rate of MGIT was very high. So we changed the sample precessing procedure and used BBL MycoPrep™, which decreased the contamination rate to one third. Cornfield et al. reperted that when specimens were processed with 4% NaOH, the contamination rate of MGIT was 29%. But when specimens were processed with 6% NaOH, the contamination rate was decreased to 12%[12]. In conclusion, the data indicates that MGIT is far superior for recovery rate and time to detection of Mycobacteria as compared to Ogawa media. MGIT could be especially more useful in isolating mycobacteria in case of extra-pulmonary specimens, such as CSF or pleural fluid, in which the number of Mycobacteria is few and rapid diagnosis is required.

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=국문요약=

MGIT와 Ogawa 배지의 결핵균 검출 비교

김영식, 조용현, 이희주, 서진태, 이영자*

경희대학교 의과대학 임상병리학교실 · Becton Dinkinson korea*

배경 : 계란기초배지인 Ogawa 배지를 이용하여 결핵을 진단하기까지는 장시간이 필요하다. 고체배지에 비해 액체배지는 진단 기간의 단축과 높은 민감도를 보여 한국에서도 90년대를 전후하여 액체배지를 사용하는 병원들이 조금씩 늘어나는 추세다. 국내에서 많이 사용되었던 액체배지 시스템은 BACTEC 460 Culture system (Becton Dickinson, Sparks, MD, USA)이었지만 방사선 폐기물 문제와 오염율 증가 문제로 인해 사용에 제한이 있었다. 방사선 폐기물을 발생시키지 않은 새로운 검사법 중에 BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA)은 형광물질이 함유된 Mycobacteria Growth Indicator Tube (MGIT)을 이용한다. 본 연구는 MGIT의 유용성을 알아보기 위해 Ogawa 배지 배양 및 항산성 도말 검사와 비교분석해 보았다.

방법 : 재료는 주로 결핵이 의심되는 환자의 객담 232 검체를 대상으로 하였으며, 검체 채취후 5일 이내에 MGIT와 Ogawa 배지에 동시에 접종하였다. 접종 후 MGIT는 6주까지, 그리고 Ogawa 배지는 8주까지 관찰하였다.

결과 : 결핵균 검출율에 있어서 MGIT가 232 검체 중 99 검체를 검출한데 비해 Ogawa 배지는 64 검체만을 검출하였다. CDC 표기에 의한 AFB 염색 정도에 따라 결핵균 검출 기간의 차이를 검사한 결과 MGIT에서는 12.6일이 소요되었고 Ogawa 배지에서는 23.7일이 소요되었다. 오염율은 MIGT에서는 5.1%, Ogawa 배지에서는 5.6%이었다.

결론 : MGIT는 국내에서 사용되고 있는 Ogawa 배지에 비해 결핵균 검출율과 검출기간 면에서 좋은 방법이라고 사료되었다.