Malaysian J Pathol 2009; 31(2) : 93 – 97

ORIGINAL ARTICLE

The manual MGIT system for the detection of *M tuberculosis* in respiratory specimens: an experience in the University Malaya Medical Centre

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Abstract

A prospective study was conducted on 510 respiratory specimens for the presence of *M. tuberculosis* detected by direct acid-fast bacilli (AFB) smear examination, culture in the Manual *Mycobacteria* Growth Indicator Tube (BBL MGIT, Becton-Dickinson) and culture on Lowenstein-Jensen (LJ) medium. From positive BBL MGIT tubes, Ziehl-Neelsen and Gram stains were performed and subcultures were put up on LJ medium. A total of 101 (19.8%) specimens were positive by the BBL MGIT, 60 (11.8%) by primary LJ medium culture, 31 (6.1%) by direct smear examination and 29 (5.7%) by all three methods. Using primary LJ culture as the gold standard, the sensitivity and specificity of the BBL MGIT were 90% and 89.6% respectively but the sensitivity of AFB smear microscopy was only 48.3%. About half (51.1%) of the BBL MGIT false positives were due to contamination by non-AFB bacteria. The remaining false positives comprised specimens that were AFB microscopy positive but LJ culture negative. Of the AFB isolates obtained on LJ primary and sub-cultures, almost all (93.3%) were identified as *Mycobacterium tuberculosis* complex. The mean time-to-detection was significantly shorter ($p<0.0001$) for the BBL MGIT than for LJ culture. For the former, positive results were available within 14 days for both AFB smear-positive and AFB smear-negative specimens. On the average, positive results were obtained 1.8 days earlier for direct AFB smear-positive samples than for AFB smear-negative samples. On the other hand, positive growth on LJ medium appeared after at least 33 days of incubation. These findings suggest that the BBL MGIT system will be a suitable alternative to LJ culture for the routine diagnosis of pulmonary tuberculosis, but a combination of liquid and solid cultures is still required for the highest diagnostic accuracy.

Keywords: *Mycobacterium tuberculosis* diagnosis, manual MGIT system

INTRODUCTION

Mycobacterium tuberculosis, the aetiological agent of tuberculosis (TB), is one of the most successful bacterial pathogens in the history of mankind. Despite the availability of anti-tuberculosis chemotherapy for the past 5 decades, this organism continues to cause extensive morbidity and mortality, infecting one third of the world's population and causing an estimated 8.8 million new cases and approximately 2 million deaths each year.[1] TB is also the primary cause of death among individuals infected by the human immunodeficiency virus. The World Health Organization (WHO) in 1993 declared TB to be a global public health emergency and reported that more than 95% of the estimated annual new cases and deaths occur in the developing world.[2]

The *M. tuberculosis* complex comprises human and bovine strains of *M. tuberculosis* as well as *M. microti, M. africanum, M. canettii, M. caprae* and *M. pinnipedii* which are very closely related genetically but can be differentiated by biochemical and molecular characteristics. Currently, all commercially available tests for TB detect the *M. tuberculosis* complex as a single entity. The *Mycobacterium* Growth Indicator Tube (MGIT) system from Becton-Dickinson was introduced about a decade ago as a means to speed

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up the isolation of slow-growing mycobacteria. This is done by liquid culture in a modified Middlebrook 7H9 broth and detection of growth by a ruthenium pentahydrate fluorescent oxygen sensor embedded in silicon at the bottom of the culture tube. Oxygen reduction induced by metabolising bacteria reduces fluorescence quenching. Hence, the level of fluorescence that the tube emits corresponds to the amount of oxygen consumed by organisms in the tube, and is proportional to the number of bacteria present.

This test is now automated for continuous monitoring in the BACTEC MGIT 960 (Becton Dickinson) but the older manual system requires tubes to be examined for fluorescence under a Wood's lamp or with some other long wave UV light source. Though requiring more technical time, the manual system is a lot more affordable for laboratories with a smaller budget. In this study, the manual MGIT system (BBL MGIT) from Becton Dickinson was evaluated against conventional culture on Lowenstein-Jensen (LJ) agar slopes and microscopical examination for acid-fast bacilli (AFB) in Ziehl-Neelsen (ZN) stained smears.

MATERIALS AND METHODS

Clinical samples

The clinical samples studied comprised sputa, bronchoalveolar lavages, pleural fluids and tracheal aspirates collected from patients suspected to be having pulmonary or pleural TB. These were all consecutive samples received in the TB and Fungus Laboratory, University Malaya Medical Centre, from March 28, 2007 until September 26, 2007. All specimens were processed for AFB smear microscopy and culture on the day of collection.

Microscopy

Smears were screened by Auramine-O fluorescence microscopy under 40x magnification. Positive smears were re-examined with ZN staining for AFB under oil immersion.

Culture on Lowenstein-Jensen medium

Regardless of microscopy results, all specimens were decontaminated and digested using the BBL MycoPrep Reagent (Becton Dickinson) containing N-acetyl –L-cysteine- 2% sodium hydroxide (NALC-NaOH). For each specimen, equal volumes (about 1 ml each) of reagent and specimen were pipetted into a centrifuge tube, vortexed briefly and allowed to stand at room temperature for 15 minutes. The BBL MycoPrep Phosphate Buffer (pH6.8) was added to the 10 ml mark on the centrifuge tube and the resulting mixture was centrifuged at 3,000 rpm for 15 minutes. The supernatant fluid was discarded. A small amount of phosphate buffer was added to resuspend the sediment which was used to inoculate a slant of LJ medium (Oxoid). All cultures were incubated at 37^0C and examined daily until the appearance of colonies resembling mycobacteria. ZN staining was carried out on the colonies to identify AFB. Confirmed AFB were sent to the TB Reference Laboratory at the National Public Health Laboratory, Sg. Buloh, Selangor, for speciation.

The time-to-detection for LJ culture was defined as the time between inoculation and the earliest reading when colonies resembling mycobacteria were noted on the agar surface. Specimens were considered culture negative if there were still no colonies formed after 8 weeks of incubation.

Culture by the manual MGIT system (BBL MGIT)

Specimens were decontaminated and digested as described above for LJ culture. A portion (0.5ml) of the processed specimen was inoculated into each BBL MGIT tube together with BACTEC MGIT Growth supplement (oleic acid-albumin-dextrose-catalase) and BBL MGIT PANTA antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). The BBL MGIT tubes were incubated at 37^0C and examined daily in a 365nm wavelength UV light source fluorescence detector (Bactec TM MicroMGIT device, Sparks, MD, Maryland, USA) up to 8 weeks of incubation. A positive and a negative control tube were included with every batch of test tubes examined. In addition, bacterial growth was detected by the presence of a non-homogenous turbidity or small grains or flakes in the culture medium. When a tube was found to be positive for bacterial growth, a portion of the tube content was removed and used to prepare two smears, one for ZN staining and one for Gram staining. If AFB were present in the ZN smear, the tube content was subcultured onto a slant of LJ medium. If organisms grew on subculture and were identified as *M. tuberculosis* complex, the BBL MGIT culture was considered a true positive. However, if AFB were not seen but the Gram stained smear showed other bacteria or fungi, the BBL MGIT culture was considered contaminated. Time-to-detection was defined

as the interval between the incubation of an inoculated tube and a positive reading in a tube whose content was also AFB smear positive.

RESULTS

During the 6-month study period, a total of 510 respiratory specimens collected from 332 patients were processed. The specimen types consisted of 457sputum samples (90.0%). 37 pleural fluids (7.0%), 10 bronchoalveolar lavages (2.0%), and 6 tracheal secretions (1.0%)

The results showed 101 (19.8%) positives by the BBL MGIT, 60 (11.7%) by LJ medium culture, 31 (6.1%) by direct AFB smear examination and 29 (5.7%) by all three methods (Tables 1 & 2). From the speciation tests carried out in the National Reference TB Laboratory, Sg. Buluh, 54 (93.3%) of the 60 isolates on LJ agar were identified as *M. tuberculosis* complex. Members of the *M. fortuitum* complex made up the remaining 6 isolates.

Sensitivity and specificity

The BBL MGIT was positive for all the LJ culture positive specimens except for 6 specimens that grew *M. tuberculosis* complex, thus giving a sensitivity of 90% (54/60) against LJ culture. In contrast, the sensitivity of the AFB microscopy was only 48.3% (29/60). Of the 54 BBL MGIT positives, 29 (53.7%) were ZN smear positive and 25 (46.3%) were ZN smear negative. The specificity of the BBL MGIT was calculated to be 89.6%, as this test was negative in 403 of the 450 LJ culture negative specimens. Of the 47 false positives defined by the absence of mycobacterial growth on LJ agar, 24 were due to obvious contamination by other aerobic bacteria, thus giving an overall breakthrough contamination rate of 4.7% (24/510) for the BBL MGIT system. In the other 23 BBL MGIT positive tubes, AFB were seen in the ZN stains done on the tube contents but the LJ subcultures were negative for mycobacteria. The corresponding primary LJ cultures of these specimens were also negative. Two specimens were positive by direct AFB smear examination but negative by both culture systems. Clinical information was not available to determine whether or not these specimens were taken after the start of anti-TB treatment.

Repeat samples

Of 107 patients who gave repeat samples (2 to 3 samples each), the BBL MGIT test was negative in 80 and positive in 27 patients. Discrepant results from consecutive cultures were seen in only 6 (5.6%) patients.

TABLE 1. BBL MGIT and LJ culture results in direct AFB microscopy positive specimens

	BBL MGIT +	BBL MGIT –	Total
LJ culture +	29	0	29
LJ culture –	0	2	2
Total	29	2	31

+ Positive result
– Negative result

TABLE 2. BBL MGIT and LJ culture results in direct AFB microscopy negative specimens excluding specimens with negative results in all 3 tests

	BBL MGIT +	BBL MGIT –	Total
LJ culture +	25	6	31
LJ culture –	47*	0	47
Total	72	6	78

+ Positive result
– Negative result

** Made up of 23 tubes that were positive in the AFB stain but negative in the LJ subculture and 24 tubes with Gram-stain showing contamination by other bacteria.*

Time to detection

The mean time to detection by culture was determined for AFB smear-positive versus AFB smear-negative specimens. With the AFB smear-positive specimens, the mean times to detection were 11.5 days (SD 3.4 days; median, 12.0 days) with the BBL MGIT system, and 31.2 days (SD 5.8 days; median 32.0 days) with LJ culture. For AFB smear- negative specimens, the corresponding times were 13.2 days (SD 5.0 days; median 13.0 days) with the BBL MGIT system, and 35.3 days (SD 5.3 days; median 34.0 days) with LJ culture. The difference between BBL MGIT and LJ culture was statistically significant ($p<0.0001$).

DISCUSSION

TB in its typical clinical presentation is often diagnosed and treated on suspicion. Laboratory confirmation is however, very important not only for the optimal management of patients and their close contacts, but also for the generation of accurate epidemiological data to be used as the foundation for appropriate and effective control strategies. The strength of the laboratory network in a country is often a direct reflection of the success of its TB control programme.

Improvements in the laboratory diagnosis of TB have focused on the reduction of turn-around time for detection and drug susceptibility testing, to allow for earlier detection of drug resistance and treatment failure. Light microscopy is rapid, inexpensive, technically simple, and specific for AFB, but will only detect open infectious cases of TB. The overall sensitivity of AFB microscopy has been reported to be 22 to 78% for respiratory specimens. While 10^6 AFB/ml of sputum usually results in a positive smear, the detection rate drops to 60% if 10^4 AFB/ml are present and to only 10% if less than 10^3 AFB/ml are in the smear.[3,4] Microscopy is frequently negative for non-respiratory specimens because of the lower bacterial load, and for NTM, because some species have cell walls that do not stain well in AFB stains.[5] Furthermore, microscopy will not discriminate between *M. tuberculosis* and other mycobacteria, nor viable from dead bacilli. Hence, microscopy is not very useful for the diagnosis of early TB, post-treatment TB and some NTM infections. However, it is of epidemiologic and clinical interest to be able to diagnose TB in patients who are AFB smear-negative, because at this stage they are less contagious and have lower morbidity and mortality.[6,7]

M. tuberculosis culture is still the cornerstone on which definitive diagnosis of TB relies. It can be performed on a variety of respiratory and non-respiratory samples, is much more sensitive than microscopy and allows the recovery of the bacteria for other studies, such as the identification of mycobacterial species, drug susceptibility testing and genotyping. Liquid cultures generally require a shorter incubation time than culture on solid media for the detection of *M. tuberculosis.*[8] Automated systems such as the BACTEC MGIT 960, ESP Culture System II, and MB/BacT ALERT 3D system enable large numbers of specimens to be processed rapidly with positive results appearing usually within 10-12 days of incubation. However, these systems have similar problems of contamination, cross-contamination and the need for subculture onto solid media for the confirmation of identity and purity. Cruciani *et al*.,[9] in a meta-analysis of 10 studies, reported that the BACTEC 960/MGIT and BACTEC 460 systems showed a sensitivity and specificity in detecting mycobacteria of 81.5 and 99.6% and 85.8 and 99.9%, respectively, but when combined with solid media, the sensitivity of the two systems increased to 87.7 and 89.7%, respectively. Both systems had a higher sensitivity for smear-positive specimens. Rates of breakthrough contamination were 12.8% with solid media, 8.6% with the BACTEC 960/MGIT and 4.4% with the BACTEC 460.

In this study, the sensitivity (90%) and breakthrough contamination rate (4.7%) of the manual BBL MGIT were better than those of the automated BACTEC systems described in Cruciani's meta-analysis but the specificity (89.6%) was significantly lower. This inferior specificity could be due to the large number of specimens that were considered false positives in the BBL MGIT because they were negative in the ZN smear and LJ primary and subcultures. Although technical error could not be ruled out, some of these apparent false positives could be due to the growth of non-tuberculous mycobacterial species that require special growth factors not provided in LJ medium, or grew better in liquid than solid media.[10] With the exclusion of these contentious results, the BBL MGIT specificity would be 92.8%. As reported by other workers [9,10] the BBL MGIT was positive more often for AFB smear-positive than smear-negative specimens and the mean time-to-detection was significantly shorter for the BBL MGIT than for LJ culture, for both AFB smear-positive and AFB smear-negative

specimens. The highest recovery rate is also obtained with a combination of liquid and solid media.

Overall, the findings in this study indicate a useful role for the manual BBL MGIT system in the routine diagnosis of pulmonary TB, especially in laboratories with a small number of specimens for TB detection. Although it is more costly and laborious, it has distinct advantages over the conventional LJ culture with respect to faster growth and better growth of fastidious mycobacterial species. Nevertheless, as shown by the 6 LJ culture positive but BBL MGIT negative specimens, a combination of solid and liquid culture systems is still required for the highest recovery of mycobacteria from clinical specimens. Furthermore, as shown by the inconsistent detection in some repeat samples, the sensitivity of culture will increase with more number of specimens cultured.

ACKNOWLEDGEMENT

The authors thank Becton Dickinson Malaysia for the supply of BBL MGIT kits and technical support.

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