Original Article

Comparative evaluation of BACTEC MGIT 960 with BACTEC 9000 MB and LJ for isolation of mycobacteria in The Gambia

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Abstract
Background: The BACTEC MGIT 960 was evaluated and compared with BACTEC 9000 MB and Lowenstein-Jensen medium for recovery rate of mycobacteria, time to detection, and contamination rate.
Methodology: 147 sputum samples obtained from patients with suspicion of tuberculosis were processed and inoculated into BACTEC MGIT 960, BACTEC 9000 MB and Lowenstein-Jensen medium using standardized procedures.
Results: BACTEC MGIT 960 detected 57.1%; BACTEC 9000 MB detected 57.8%; and LJ medium detected 43.5% specimens with *Mycobacterium tuberculosis* complex (MTBC). BACTEC MGIT 960 had the shortest mean number of days (10.3) to detection, followed by BACTEC 9000 MB (13.2) and LJ medium (26.1). Sign rank test showed all three methods had significant difference in days to detection (each $P<0.0001$). About 39% of detection by BACTEC MGIT 960 took place within the first week, compared to 27.0% and 0.0% by BACTEC 9000 MB and LJ medium respectively. The best yield was obtained with BACTEC 9000 MB, but when compared with the BACTEC MGIT 960, it was not statistically significant. Performances were the same when the combination of a liquid plus a LJ medium were measured ($P=0.05$). Contamination rates were significantly higher in BACTEC MGIT 960 (12%) than in BACTEC 9000 MB (7%) ($P=0.041$) and LJ (4%) medium ($P=0.022$). BACTEC 9000 MB and LJ medium have lower contamination rates ($P=0.607$).
Conclusions: BACTEC MGIT 960 had a shorter time to detection of MTBC than BACTEC 9000 MB and L J medium. Despite a higher contamination rate, its performance did not appear to be inferior.

Key Words: *Mycobacterium tuberculosis complex*, BACTEC 9000 MB, BACTEC MGIT 960, TB clinical diagnosis.

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Introduction

Tuberculosis (TB) globally causes approximately 2 million deaths per year and remains the leading infectious cause of death in humans [1, 2]. High rates of HIV infection in sub-Saharan Africa have fueled a rise in the incidence of TB [3]. While the incidence of sputum positive tuberculosis in The Gambia is approximately 80/100.000/ year [4], the HIV co-infection rate among new smear positive TB cases was relatively low at 7% in a study conducted in 2002-2003 [5]

Developing countries usually rely on acid-fast staining, which is the most rapid method currently employed worldwide [6,7] or cultures of mycobacteria in conjunction with assessment of clinical symptoms and radiographic evidence to diagnose TB [8]. Detection of mycobacteria by acid-fast staining and culture lacks sensitivity, particularly in cases of sputum negative disease [6-8]. Extra-pulmonary tuberculosis presents even more problems, as sputum samples are often not available and obtaining specimens often involves highly invasive and expensive procedures [8].

The gold standard for TB diagnosis is the cultivation of mycobacteria on solid and/or liquid based media [7]. Conventional methods relied on egg-based (Lowenstein-Jensen) and agar-based (Middlebrook agar) media, but these are laborious and time-consuming procedures requiring from 3 to 8 weeks to obtain results [7]. The BACTEC 460 TB System (Becton Dickinson, Microbiology System, Sparks, MD) was the first semi-automated radiometric system introduced in the 1980s for rapid detection of mycobacterial growth [9] and had been the benchmark for rapid detection of *M.*

tuberculosis complex. Although very rapid and sensitive, its universal application was not achieved because of safety issues associated with radiolabelled waste disposal [10]. Fully automated non-radioactive systems have now been designed with the aim of reducing the delay in the detection of mycobacteria and improving growth rates. These include MB/BacT (Organon Teknika, Turnhout, Belgium), ESPII (Difco Laboratories, Detroit, Michigan), BACTEC 9000MB (Becton Dickenson Microbiology System, Sparks, MD) and BACTEC MGIT 960 (BBL Becton Dickinson Microbiology Systems, Cockeysville, MD) [11-15]. These systems are expensive and their implementation can be justified only for laboratories with a substantial volume of work [16].

We have not found comparative studies evaluating the performance of BACTEC 9000MB, BACTEC MGIT960 and Lowenstein-Jensen (Solid LJ) medium in a routine clinical setting in Africa, although the BACTEC MGIT960 has been compared with the BACTEC 460 TB system and Lowenstein-Jensen in developed countries. Consequently, we evaluated the fully automated BACTEC MGIT 960 system for the detection of mycobacteria in clinical specimens and compared the results with those of BACTEC 9000MB system and solid LJ medium in terms of recovery rate, mean time to detection, and contamination rate.

Materials & Methods

Specimens

Consecutive sputum samples from suspected TB patients from the MRC Outpatient Department and the major Gambia Government TB clinic in Greater Banjul were included in this study after informed consent. One hundred and forty-seven respiratory specimens (sputa) were obtained from 24 July to 22 November 2006.

Direct microscopy and decontamination procedure

For direct microscopy, smears were stained with auramine and the Ziehl-Neelsen (ZN) as previously described [17]. Respiratory specimens were digested and decontaminated using N-acetyl-L-Cysteine-NaOH. Smears were prepared for acid-fast bacilli (AFB) staining from the concentrated sediments by Ziehl-Neelsen stain as previously described [17].

Culture systems

(i) BACTEC MGIT 960. The BACTEC MGIT 960 culture tubes contain 7mL of Middlebrook 7H9 base, to which was added an enrichment supplement containing oleic acid, albumin, dextrose, and catalase (BBL OADC) and an antibiotic mixture of polymyxinB, amphotericinB, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA) as described in the manufacturer's instructions (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). After inoculation of each tube with 0.5 mL of the processed specimen, the tubes were incubated at 37^0 C in the BACTEC MGIT 960 instrument and were monitored automatically every 60 minutes for increase of fluorescence. The cultures were tested until positive or for 6 weeks.

(ii) BACTEC 9000 MB. Each BACTEC 9000MB MYCO/F culture vial (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) contains 40 mL of broth supplemented with 2 mL of an antimicrobial mixture (PANTA) consisting of polymyxinB (400,000 mg/mL), amphotericinB (70mg/mL), nalidixic acid (280 mg/mL), trimethoprim (70 mg/mL), and azlocillin (80 mg/mL). Lyophilized PANTA was reconstituted with 10 mL of supplement F as described in the manufacturer's instructions (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). The BACTEC MYCO/F vials were inoculated with 0.5 mL of the processed sediments and were incubated for 6 weeks. Since fluorescence levels must be measured every 10 minutes by the BACTEC 9000MB system, the vials were almost continuously monitored for the growth of AFB.

(iii) Solid LJ media. 0.25 mL of the processed specimen was inoculated onto one slope each of LJ medium containing glycerol and sodium pyruvate respectively. All solid media were incubated at 37^0 C and inspected weekly for 8 weeks or until colonies were detected.

Culture protocol

For all systems, when the first culture medium was identified as positive, an AFB smear (ZN) was performed the same day. If the smear was prepared from a liquid medium and it was positive for AFB, the liquid medium was subcultured on L J for identification by means of conventional biochemical tests [5, 26] and on Blood Agar and Sabouraud Dextrose Agar to check for contaminants. If the smear was performed from an

LJ slant and it was positive for AFB, no further subculture was needed. When either liquid medium was signaled as positive, a sample of the broth was removed aseptically and used to prepare a smear stained for AFB and subcultured on Blood Agar and Sabouraud Dextrose Agar to check for contaminants. If AFB were present in the smear, a portion of the broth was subcultured on L J for identification by means of conventional biochemical tests. If both AFB and other organisms were present, the broth was re-decontaminated and cultured again. The time to detection (TTD) was based on the earliest time of positivity recorded by instrument that correlated with the positive AFB smear result. If the smear was negative for AFB, a smear of broth was stained with the Gram stain. If bacteria or yeast was present, the culture was considered contaminated and was discontinued. Contaminants were identified by standard bacteriological methods.

Growth on solid media was detected by visual observation with hand lens for mycobacterial colonies. The TTD was based on the earliest date of detection of colonies on any of the solid media. All growth was confirmed by making smears and staining for AFB. If no growth occurred by the end of the week 8, the culture was considered negative for mycobacterial growth.

Randomly selected instrument negative BACTEC 90000MB vials and BACTEC MGIT 960 tubes (at the end of 42 days protocols) were examined for AFB and subcultured on solid LJ media. No microorganisms were detected by smears or by subsequent cultures. No false negative samples from these random terminal subcultures were detected during the study.

Identification of mycobacteria

Identification was based on colony morphology, pigmentation, rate of growth on conventional solid media, and standard biochemical test methods [5]. The TB Laboratory submits to the UK National External Quality Assessment Scheme for Microbiology and TB and to the WHO supra-laboratory quality assurance for TB drug susceptibility testing.

Statistical analysis

Kappa statistics were used to assess agreement between each pair of methods. Non-parametric procedures were used in the statistical comparisons throughout [18]. The sign test was used to assess difference in proportions between results from a pair of methods applied to the same specimens. The sign-rank test was used to assess difference in number of days to the detection of MTBC or contamination between results from a pair of methods applied to the same specimen. The Mann-Whitney test was used to compare days to detection between smear positive and smear negative samples. We considered specimens with both culture and smear negative as true negative and used these for the assessment of specificity (sensitivity) of the two automated systems.

Results and Discussion

All positive isolates were confirmed as *M. tuberculosis* (MTB). Of the total MTB isolates recovered, 85 (57.8%), 84(57.1%), and 64 (43.5%) of the specimens were detected by BACTEC 9000 MB, BACTEC MGIT 960, and LJ medium respectively (Table 1). This result implied that 14.3% and 13.6% additional MTB isolates were detected after BACTEC 9000 MB and BACTEC MGIT 960 systems were incorporated respectively. The increased yield of BACTEC 9000 MB and BACTEC MGIT 960 systems over LJ medium may have been due to the recommended inoculum size (0.25 ml for LJ and 0.5ml for the BACTECs), addition of enrichment supplements and PANTA, and continuous monitoring. Both automated systems were significantly more likely to yield a positive result than with the LJ medium, whereas the two automated liquid systems did not differ significantly (Table 1).

The BACTEC MGIT 960 system agreed with the LJ medium slightly less well (Kappa=0.699, P<0.001) than with the BACTEC 9000 MB system (Kappa=0.746, P<0.001). Among the 147 specimens, culture and smear agreed on 136 (63 both positive and 73 both negative). In addition, the sensitivity for the BACTEC MGIT 960 system and the BACTEC 9000 MB system was 100% and 98.4% respectively, and the specificity was 97.3% and 95.9% respectively, similar to other reports on mycobacteria cultures from clinical samples [19, 20].

Recovery rates of MTBC from BACTEC 9000 MB and BACTEC MGIT 960 systems were significantly higher than those from LJ medium (P<0.05 and P< 0.01) respectively, similar to

previous studies based on comparisons of different liquids with solid medium [19, 21, 22]. In this study, the BACTEC MGIT 960 system displayed a rate of recovery (94.6%) of MTBC higher than those previously reported from the same system (81.4% by Pfyffer *et al*. [19]; 77% by Hanna *et al*.; [20] and 84.6% by Natalie *et al*. [23].

In addition, the BACTEC MGIT 960 system had a shorter time to detection of MTBC than BACTEC 9000 MB system and LJ medium (Table 1).The difference between the two automated liquid media was not statistically significant. The time to detection with the BACTEC MGIT 960 system was 2.3 days shorter than the time of 12.5 days reported by Tortoli *et al*. [24]. Regardless of the culture systems, the mean number of days to detection was slightly longer for specimens that were initially smear negative but this was not statistically significant according to the Mann-Whitney test (each P>0.05).

Table 1. Summary of test systems/methods results in relation to different findings among the (147 sputa).

Aspect	Findings in testing by:		
	BACTEC 9000 MB	BACTEC MGIT 960	Solid LJ
MTBC yield/recovery (%)	85 (57.8)	84 (57.1)	64 (43.5)
Mean (range) days to detection	13.2 (4-33)	10.3 (2-34)	26.1 (14-56)
Detection within the first week (%)	24 (27)	34 (39)	0
Contamination rate (%)	10 (7)	17 (12)	6 (4)

Bacteria contamination appeared in 33 of 147 specimens (data not shown). The contaminants identified include coagulase negative *Staphylococci*, *Pseudomonas aeruginosa*, *Candida* species, and *Bacillus* species. The overall rates of contamination were found to be higher with BACTEC MGIT 960 system than with BACTEC 9000 MB system and LJ medium (Table 1). The contamination rates for the BACTEC MGIT 960 system in this study were also much higher than those reported in other studies (8.1% by Hanna *et al*., [20] and 10.0% by Tortoli *et al*.,[24]). In contrast, the rates were lower than 17.1% reported by Natalie *et al*. [23].

The combinations of each liquid media with LJ displayed slightly lower rates of recovery of MTBC; there was no statistically significant difference between the two combinations. The combination of two liquid media was, however, more efficient in isolating MTBC than the use of the gold standards (Combination of liquid and solid media). The differences were statistically significant, in contrast to findings by other workers [19, 25, 26].These data suggest that a combination of two liquid media may be more attractive than the use of a liquid plus a solid media (the traditional gold standard). Such an approach is limited by cost.

Both the BACTEC 9000 MB and BACTEC MGIT 960 systems offer certain advantages. These include computer-based data management capabilities, which simplify tracking of results and can be interfaced with a laboratory's information system; no radioactive waste disposal cost; adaptability for drug susceptibility testing; less labour intensive mycobacterial culture system; and reduced risk of cross-contamination [14]. With regard to the BACTEC MGIT 960 system, the culture tubes are plastic rather than glass, and they have screw caps, thus eliminating the need to use needles for the addition of supplements and specimen inoculation, and maximum capacities [23]. The time to detection may be biased toward the BACTEC 9000 MB and BACTEC MGIT 960 systems because their continuous monitoring provides an unequivocal advantage in comparison to the weekly detection with the conventional method. Other differences, inoculum size and culture medium may account for the different rates.

Conclusions

Recovery rates of MTBC from the two automated systems had similar performance in terms of agreement with solid medium, sensitivity and specificity. The BACTEC MGIT 960 system had a shorter time to detection of MTBC than BACTEC 9000 MB system and Solid LJ medium. The BACTEC 9000 MB system had lower contamination rates but is no longer marketed and has been replaced by the BACTEC MGIT 960. Although further studies to reduce contamination rates are required, we recommend that an automated liquid culture system be used in reference laboratories in developing countries as stipulated by WHO [27] although some low-

income, developing countries may not be able to afford this.

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